Marine and Coastal

National Environmental Science Program

13. Microplastics

Nina Wootton*[1], Patrick Reis-Santos*[2], Tanveer Adyel, Michelle Blewitt, Brad Clarke, Thomas Crutchett, Mark Hamann, Denise Hardesty, Jennifer Lavers, Sophie Leterme, Frederic Leusch, Sam Lynch, Cherie Motti, Allyson O'Brien, Elvis Okoffo, Kushani Perera, Peter Puskic, Joseph Razzell Holls, Lauren Roman, Marina F. M. Santana, Anastasiia Snigirova, Elise M. Tuuri, Scott Wilson, Shima Ziajahromi, Bronwyn M. Gillanders*[3]

[1]nina.wootton@adelaide.edu.au
[2]patrick.santos@adelaide.edu.au
[3]bronwyn.gilllanders@adelaide.edu.au

Chapter citation:.
Wootton N, Reis-Santos P, Adyel T, Blewitt M, Clarke B, Crutchett T, Hamann M, Hardesty D, Lavers J, Leterme S, Leusch F, Lynch S, Motti C, O'Brien A, Okoffo E, Perera K, Puskic P, Razzell Holls J, Roman L, Santana MFM, Snigirova A, Tuuri EM, Wilson S, Ziajahromi S, Gillanders BM. 2024. Marine sampling field manual for microplastics. In *Field Manuals to Monitor Australian Waters, Version 3.* Przeslawski R, Foster S (Eds). National Environmental Science Program

Introduction and Scope

Introduction

An increasing number of organisations are undertaking research, monitoring, and data collection on the presence of plastic contamination in the environment. This information is essential for establishing baseline data to support the establishment of indicators and targets for decision-making to mitigate plastic contamination.

Recognising the imperative need for improved data harmonisation, there is a requirement to develop standardised approaches to allow reliable data comparison. This includes developing sets of reproducible practices and guidelines, covering all steps from sample collection, processing, laboratory procedures, and plastic characterisation. It also includes ensuring consistent terminology and data reporting parameters.

This manual is part of a suite of marine sampling field manuals that aim to facilitate reliable data comparisons across regions, and national and international collections, by implementing reproducible and comprehensive guidelines, covering all steps from sample collection, processing, laboratory procedures, and plastic characterisation. The approaches in these manuals provide methods for collecting data that are endorsed by researchers, managers, and technicians from multiple agencies with a variety of experience and subject-matter expertise. The manuals follow the FAIR Guiding Principles for scientific data (Wilkinson et al., 2016), aiming to be findable, accessible, interoperable and reusable. See Chapter 1 and Przeslawski et al. (2023) for a description of the process undertaken to develop these manuals.

This manual focuses on microplastics in water, sediment, biota and air matrices, and spans everything from sampling design, sample collection, processing and laboratory procedures, and plastics characterisation (i.e., size, type, polymer composition). It establishes recommended terminology and makes a distinction between essential and desirable data reporting parameters needed to guarantee accurate, efficient and standardised approaches to microplastic analysis in the environment. It also includes a checklist to facilitate reporting. Aligned with international guidelines, such as those set by the Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP) (GESAMP, 2019) the manual is divided into four sections, each dedicated to a specific environmental matrix (Sediment, Water, Air, and Biota). Such information is critical for establishing consistent guidelines in the collection and processing of microplastic data from different environments, enabling scientifically robust comparisons between studies, sites, projects, and organisations at a national level.

What are microplastics?

Microplastics are defined as "*any synthetic solid particle or polymeric matrix*, *with regular or irregular shape and with size ranging from 1 µm to 5 mm*, *of either primary or secondary manufacturing origin*, *which are insoluble in water*" (Frias and Nash, 2019).

Scope, format and purpose

The manual focuses on the identification and quantification of microplastics between 1 µm and 5 mm, with the caveat that smaller pieces, namely between 1 µm and 20 µm, require added specialised approaches. Though many researchers find it useful to delineate groupings of plastic size (i.e., nano, micro, meso, and macro plastics), it is important to note that plastic size exists on a continuum. The chosen size categories are based on widely available methodologies to sample, characterise and identify microplastics. While the methods presented here are also appropriate for larger plastic debris (>5 mm), the described processes are designed for microplastics (<5 mm). Analysis of nanoplastics below the set threshold of 1 µm requires increasingly specialised approaches and quality controls that would warrant independent and targeted field manuals in their own right, and are thus not included here.

Due to the differences in methods required to sample microplastics across the defined size range between 1 µm and 5 mm, this manual is divided into sampling, identifying, and reporting microplastics in two main size tiers. These include those that are visible to the naked eye (1 mm – 5 mm; visible microplastics) and those that are microscopic (1 µm – 1 mm; microscopic microplastics).

This tiered approach allows research groups and organisations to accurately identify microplastics depending on the research question, level of experience, available facilities, and budget. It is an inclusive strategy to ensure microplastic sampling can occur accurately and comparably between NGOs, consultants, research and government institutions.

These manuals are designed to deliver improved data collection across surveys and collectors with different levels of background expertise and knowledge. Several sections refer to essential methodological steps and reporting parameters needed that allow data integration across different approaches (e.g., from citizen science to scientific research to government monitoring). To identify microplastics in the smaller size category (1 µm – 1 mm), there is a higher level of analysis that must be followed, and subsections throughout the manual highlight how and where this further detail is required.

A checklist is also provided as a quick reference guide to enhance reproducibility, promote consistency, and facilitate uptake and reporting.

Glossary of terms

Contamination:

In the context of microplastic research, contamination is the unintentional introduction of microplastic particles from uncontrollable factors, or those that despite best efforts to control them may still impact the accuracy and reliability of the results. Substantial efforts are made to mitigate microplastic contamination of samples during sample collection and processing steps to enhance the precision and credibility of results (including the use of procedural controls).

Controls:

Deliberate measures implemented to minimise and account for potential sources of microplastic contamination.

Macroplastics:

Relatively large plastics, typically exceeding 2.5 cm in size, that can be visually identified as plastic products.

Mesoplastics:

Intermediate-sized plastic particles, ranging from greater than 0.5 cm to 2.5 cm. These are smaller than macroplastics but are generally discernible to the naked eye.

Microplastics:

Small plastic particles, typically less than 0.5 cm (i.e., < 5 mm) but greater than or equal to 1 µm in size, which are manufactured at a small scale (primary microplastic) or originate from the degradation of larger plastic items (secondary microplastic). Due to the small size of microplastics, these are mostly not discernible to the naked eye.

Nanoplastics:

Plastic particles that are smaller than microplastics with dimensions in the nanometer range. These are typically less than 1 µm (< 1000 nm). Nanoplastics can be intentionally manufactured at the nanoscale (primary nanoplastics) or result from the breakdown of larger plastic items (secondary nanoplastics). They pose unique challenges in terms of detection, characterisation, and understanding their environmental and biological impacts due to their nano-scale size.

Plastic morphology:

The description of the physical structure, form and characteristics (such as size, type, and colour) of plastic particles. The morphology of plastic artefacts influences factors such as the movement, distribution, transportation, ingestion capability, and environmental impacts of the plastic contaminant.

Polymer:

The core chemical composition (i.e., building blocks) of plastic materials. Polymers are large molecules composed of repeating monomers. Polymer composition, together with the presence of different molecular groups, define the main chemical properties of plastics. These include attributes and behaviours associated with chemical stability, toxicity, solubility, chemical resistance, degradation, and the presence and behaviour of additives.

Primary Plastic(s):

Plastics intentionally manufactured in a controlled size (macro, meso, micro, and nano) and shape for specific, predetermined purposes. These plastics are produced with a defined application, distinguishing them from secondary plastics. The intentional design and production of primary plastics allow for customisation to meet specific functional, aesthetic, or industrial requirements.

Secondary Plastic(s):

Plastics generated through the degradation or breakdown of pre-existing primary plastics (macro, meso, micro, and nano), resulting in the formation of new particles across macro, meso, micro, and nano sizes. These secondary plastics are distinct from primary plastics, as they originate from the recycling, weathering, or other processes acting upon primary and/or secondary plastic materials.

Spectroscopy:

A method for the identification of plastic polymers. Spectroscopy is an analytical technique that assesses the interaction of matter with electromagnetic radiation, resulting in a unique spectrum (specific for each matter) that is used for identifying the polymer(s) of the plastic.

Quality Assurance (QA):

Planned and systematic process that ensures the reliability, reproducibility, accuracy, and precision of data through the establishment and implementation of standardised procedures, protocols, and measures.

Quality Control (QC):

Mechanisms, measures and procedures taken to ensure the reliability and accuracy of sampling and analytical results.

Weathering:

The gradual alteration of plastic particles through physical, chemical or biological processes which alter the physical and chemical properties of plastic particles over time.

Abbreviations

Abbreviations of General Terms

MP(s)	Microplastics
NP(s)	Nanoplastics

Abbreviations of Common Polymers

PA	Polyamide
PE	Polyethylene
PET	Polyethylene terephthalate
PMMA	Polymethyl methacrylate
PP	Polypropylene
PS	Polystyrene
PVA	Polyvinyl alcohol
PVC	Polyvinyl chloride
PU	Polyurethane

Abbreviations of Methodologies and Chemicals

FTIR	Fourier Transform Infrared Spectroscopy
LDIR	Laser Direct Infrared Spectroscopy
Raman	Raman Spectroscopy
Py-GC/MS	Pyrolysis Gas Chromatography Mass Spectrometry

H_2O_2	Hydrogen Peroxide
KOH	Potassium Hydroxide

Contextualising this field manual with other global efforts

This manual adds to a number of guidance manuals for research and monitoring of plastic debris in marine environments. These include general guidance for research design, collection and reporting protocols across multiple habitat types (including biota) and are designed to be applicable across broad geographies (i.e. regional jurisdictions). However, most of these manuals and guidance documents do not specifically focus on microplastics, though they include this size category within their broader framework of plastics reporting.

Notable manuals or guidance documents include:

1) Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP): Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean (2019).

The Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection (GESAMP)'s "Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean" is the premier guidance document, compiled by international experts for designing marine debris monitoring and research programs, with a focus on litter of all sizes, including microplastics. Its main purpose is to provide recommendations, advice and practical guidance, for the establishment of programs to monitor and assess the distribution and abundance of plastic litter, also referred to as plastic debris, in the ocean. This guidance document does not prescribe specific one-size-fits-all methods but rather presents a menu of different approaches and guidance for different research questions, covering seawater, sediment and biota. The GESAMP document intends to promote a more harmonised approach to the design of sampling programs, the selection of appropriate indicators (i.e. type of sample), the collection of samples or observations, the characterisation of sampled material, dealing with uncertainties, data analysis and reporting the results and also to inform the establishment of national and regional field monitoring programs.
Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean | GESAMP

2) National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program: Marine Debris Monitoring and Assessment Project (MDMAP) (2021).

The NOAA Marine Debris Program coordinates the Marine Debris Monitoring and Assessment Project (MDMAP), a NOAA citizen science initiative that engages NOAA partners and volunteers, and this 2021 program builds upon earlier iterations with similar goals. This program is based in the United States but these guidelines are widely used

around the world by numerous countries, especially in the Americas. The MDMAP has a macro litter and shoreline focus and includes items >2.5cm. Items less than 2.5cm (including visible sized microplastics) are neither recommended to be counted nor characterised, and the MDMAP does not include a specific method for smaller items.
NOAA Marine Debris Monitoring and Assessment Project Shoreline Survey Guide | OR&R's Marine Debris Program

3) National Oceanic and Atmospheric Administration (NOAA) Marine Debris Program: Laboratory Methods for the Analysis of Microplastics in the Marine Environment (2015).

To complement its marine debris program, NOAA published a set of standard methods for the assessment of microplastics in the laboratory in 2015. The "Laboratory Methods for the Analysis of Microplastics in the Marine Environment: Recommendations for quantifying synthetic particles in waters and sediments" are a popular protocol for sampling microplastics across environments, and though they were designed for seawater and sediment, these methods can be used for other matrices (e.g., biota). However, since their publication, new techniques for microplastic quantification and characterisation have been developed and adopted.
Laboratory Methods for the Analysis of Microplastics in the Marine Environment | OR&R's Marine Debris Program (noaa.gov)

4) Guidance on the Monitoring of Marine Litter in European Seas (2023).

This 2023 "Guidance on the Monitoring of Marine Litter in European Seas" is an update to improve the harmonised monitoring of marine litter under the Marine Strategy Framework Directive, and builds on the 2013 version of "Guidance on the Monitoring of Marine Litter in European Seas" (2013 version -> JRC Publications Repository - Guidance on Monitoring of Marine Litter in European Seas (europa.eu).
This guidance document includes general approaches and prescribes standard methods for beaches, seawater, seafloor and biota, with variations for different size classes "macrolitter" (items >25mm), "mesolitter" (items 5-25mm) and microlitter (items <5mm). This methodology is broadly used by institutions within the European Union member states.
(PDF) Guidance on the Monitoring of Marine Litter in European Seas (europa.eu)

5) Commonwealth Scientific and Industrial Research Organisation (CSIRO) Global Plastic Leakage Baseline Project (2020)

Of particular note within the Australian context, the CSIRO Global Plastic Leakage

Baseline Project prescribes a harmonised, stratified and representative standard methodology for monitoring marine debris across shorelines, on riverbanks, inland and at the sea surface. This standard methodology builds on earlier iterations, and has been designed to harmonise with many of the other global marine litter protocols, such as the NOAA MDMAP. This methodology records items of all visible size ranges, including microplastics that are visible to the naked eye. It has been used in all states and territories throughout Australia, and approximately 20 countries worldwide.
Resources – Marine Debris Research (csiro.au)

Identifying research goals

Gone are the days when research was conducted in silos. This is especially the case with transboundary environmental problems, such as the contamination of terrestrial, aquatic and marine environments with microplastics. Data collection in the modern age is not just the domain of scientists, government workers and other professions, but also members of the public and community organisations - often referred to as 'citizen scientists'. People from all walks of life are working together with the collective goal of understanding and managing pollution in our shared environment. To this end, clearly identifying the goals of the research or monitoring project, and determining who will be the likely users of the information generated, are important first steps before embarking down the path of sample and data collection.

Common immediate goals might include investigating:

- What is the accumulation of microplastics in a specific environmental compartment or geographic area?
- Are there variations in microplastic accumulation in space or time?
- What are the sources (provenance) and fate of environmental microplastic?
- What are the potential effects and risk of microplastics?
- How many and what type of microplastics are organisms consuming?
- How are organisms impacted when consuming microplastic?

End users of the collected data might include:

- You and other members of your research team, university or organisation conducting research, teaching or community engagement activities.
- Other universities or organisations seeking to compare findings.
- Community groups, advocates and politicians seeking to understand microplastics in their local context and drive change.
- Local, state and federal levels of the Australian government seeking to measure, monitor or manage microplastic in their jurisdictions.
- Stakeholders aiming to assess the success of implemented actions (punctual or diffused)

to help address microplastic contamination.
- Businesses seeking to understand the impacts of microplastic contamination and make changes to their operations.
- International or multilateral research groups, intergovernmental panels and non-government organisations seeking to understand the scale of microplastic issues and identify targets for management.

Researchers should also consider Indigenous leadership and collaboration as related to Sea Country.

Because the goals of data collection, studied habitats and end users of information are so diverse, different groups often "speak different languages" when it comes to microplastic sampling, including laboratory procedures, quality control and data reporting, which potentially leads to a loss in research value (e.g., time and resources invested in data collection) and an inability to leverage data usage, limiting spatial and temporal comparisons of microplastics.

Harmonisation is a concept that acknowledges methodological differences between studies while also ensuring data are collected and reported according to a set of essential, minimum standard criteria and workflows, to enable groups with different goals to generate transferable information. Not all existing methods are scientifically robust, therefore before embarking on new sample and data collection activities, we recommend identifying appropriate methods that will support harmonisation of outputs. This document presents methods that will increase the comparability of the data you collect with other microplastic information gathered in Australia and across the world.

Field manual for observing microplastics

In the remainder of this section, we present general methods for observing microplastics in marine and coastal environments. This includes information on the sampling design, equipment and materials required for collection and post-survey procedures for physical and chemical characterisation. More specialised methods are described in the water, sediment, biota and air sections.

Figure 1 below shows a quick guide that can be followed when designing sample collection for microplastics and what metrics to include to ensure data harmonisation and comparability. Additionally, we have included a checklist at the end of this section that should be used when collecting, processing, and reporting data on microplastics in coastal and marine environments.

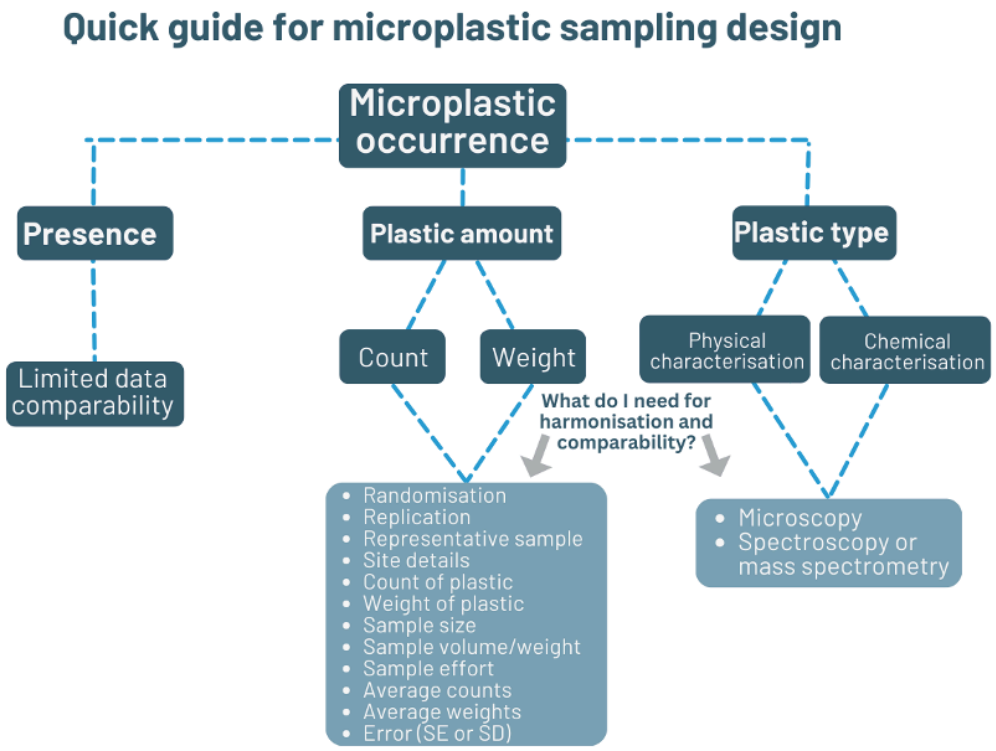


Figure 1. A quick sampling design guide for collecting data on microplastic presence, amount and type, with the metrics required to achieve data harmonisation.

Pre-survey preparations and field procedures

These methods will differ among environments so refer to the relevant section:

Water

Pre-Survey Preparations

The methods for the collection of microplastic in marine and coastal waters can have minor variations tailored to specific water compartments i.e., surface, sub-surface and at depth. These methods may also be adapted to collect microplastics within a specific size range.

Sampling design

Before commencing field sampling, it is essential to formulate the research question(s) (see: What are the goals of my research?). Different research questions may require different sampling designs and, likely, different sample collection methods. Furthermore, sampling designs should consider the post-survey requirements, including the time required to accurately process samples, the techniques to be used to analyse them and the units of reporting.

The next step is to define the spatial and temporal extent of the research question. Spatial studies can provide information on the presence of and changes in the distribution of microplastics across sites. Therefore, the sampling design must consider the number and spatial dispersion of the collection sites to answer the research question, site accessibility, and environmental characteristics that may influence results (e.g., the hydrodynamic and depth profile to be surveyed and the expanse of the water body). Temporal studies can provide information on changes in microplastic concentrations at a specific location over time; the sampling design must therefore consider site accessibility across seasons and during/after significant weather events.

Research conducted across spatial and/or temporal scales requires a stratified sampling design that enables an estimate of the true microplastic density (or type, weight, volume) at different scales or "strata" representing clearly defined groups (Quinn and Keough, 2002). Samples should be collected randomly from each group. Stratified sampling should also be used when within-habitat variation is high, and it is not feasible to sample entire sites (CSIRO 2022). Refer to the Survey Design SOP for further details.

Sampling design and approach is dependent on study objectives and will define limitations on the size class of microplastics that can be analysed. For water, sampling can be performed using tow nets, Niskin bottles, submersible pumps, underway vessel pumps or

grab samples - see descriptions below. Each mode of water sampling offers a different capability (e.g., in situ filtration of large volumes vs collection of smaller volumes for later processing; the choice being dictated by downstream detection thresholds) and different representativeness (e.g., horizontal vs vertical gradients of the water body, or specific microplastic sizes or types due to density), both of which need to be considered to ensure the research question can be satisfactorily answered (GESAMP, 2019). For example, many tow nets have a 350 µm aperture so smaller microplastics would only be opportunistically sampled, while submersible pumps, Niskin bottles and underway vessel pumps can collect microplastics across the full size range, including smaller microplastics.

The number of samples collected will be determined by the research question as well as the budget, logistics and resources available; with the caveat that a high number of samples allows for greater accuracy (through power and replication) in the estimation of microplastic concentrations in the environment and will ensure greater statistical rigour. It is important to consider what would be an appropriate effect size (i.e. the acceptable difference between the estimate and the true density or volume of microplastics in the water). Before commencing sample collection, a power analysis can be performed to assist in this decision. G*Power (Faul et al, 2007) or pwr package in R (Champley et al., 2020) are suitable for simple sampling designs and can determine the number of samples required to achieve the desired effect size.

Field Procedures

Materials and equipment for collection

The materials and equipment required for collection of water samples varies depending on the type of sampling (Table 1). For volume reduced net tows, submersible pumps and bulk water, the aperture of the filter or net must be recorded. To accurately record the volume of the water sampled a flow meter is required for net and pump devices. GPS values and depth measurerments provide additional important contextual information on the site location and details.

Table 1. List of field equipment and materials needed for collection of environmental samples in marine and coastal habitats. Three common methods are considered: volume-reduced net tow filtration, submersible pump filtration and bulk water bottling. Asterisks* indicate essential items.

Volume reduced net tow filtration	Submersible pump filtration	Bulk water bottling
Nets (e.g., Manta trawl, Neuston net, AVANI trawl, Bongo net, Hydra-bios nets)*. Net aperture and mesh size must be considered and recorded	Pump device (e.g., built-in underway seawater intake system, portable pump; including pumps deployed in a Rosette device)*. Filter aperture must be considered and recorded.	Sampling bottle of known volume (e.g., glass or plastic bottle of 1 L or more, Niskin and Van-Dorn bottles, including deployed in a Rosette device)*. Volume of sampling bottle must be considered.
Flow meter	Flow meter	Volume of water from sampling bottle must be considered and recorded
GPS start and end coordinates*	GPS start (and end when applicable) coordinates*	GPS*
Depth measurer	Depth measurer	Depth measurer
-	Metal sieves (depending on the pump design)	Metal sieves (depending on the sampling bottle)
Sampling jar*	Sampling jar*	Sampling jar
Labels*	Labels*	Labels*
Field observation datasheet*	Field observation datasheet*	Field observation datasheet*
Pencils, permanent marker*	Pencils, permanent marker*	Pencils, permanent marker*
Metal forceps and/or tweezers	Metal forceps and/or tweezers	Metal forceps and/or tweezers
Reagents (ultrapure water, ethanol)	-	-

General principles

Ocean and coastal environments are dynamic and are inherently highly variable, so it is recommended to sample over time (e.g., seasonally) if feasible, and related to research question (GESAMP, 2019). The rate and extent of seasonal change is a key factor to consider when sampling in the environment. In Australia there are typically four seasons in temperate regions (summer, autumn, winter and spring) or two in tropical regions (wet and dry), but these may be further split based on the local microclimate and different cultural group definitions. When sampling over time is not feasible, recording and reporting information on weather and other environmental conditions at the time of sample collection is crucial for contextual information (see Reporting section).

Airborne and handling contamination is likely to occur during field sampling, so appropriate quality assurance and quality control (QA/QC) procedures must be consistently applied throughout sample collection (see Quality assurance and quality control section). This includes the use of positive controls and blanks during sample collection.

Volume reduced net tows

The survey of water at the surface, sub-surface or at depth, requires the use of compartment-appropriate nets. The methods highlighted below are recommended for surface sampling although can be adapted for mid-column or near ocean floor sampling (Eriksen et al., 2018).

The most commonly used nets to assess surface water are the Manta trawl and the Neuston net, both towed from the side of the vessel, ideally downwind and at a speed of up to 4 knots (Kroon et al., 2018; Viršek et al., 2016). Ideally, sampling should be conducted during calm weather conditions. Manta trawls generally sample the top 15-25 cm while Neuston nets sample the top 50 cm, but both are considered surface tows. Another commonly used net is the AVANI trawl, generally deployed when surveying longer transects as the net can be towed at up to 8 knots. All three net types are relatively inexpensive and simple to operate. Data from all three trawl methods are comparable, as similar types (i.e., positively buoyant) and amounts of microplastic are collected. Whilst reporting information on net configuration is recommended, details on the mesh aperture size are essential and must be recorded against the sample as it defines the lower size category of collected microplastics (e.g., if mesh size is 330 µm, then microplastics greater than 330 µm will predominantly be collected and every item smaller than that should be considered as opportunistically sampled) (Hidalgo-Ruz et al., 2012).

Net deployment procedures need to be tailored to avoid airborne contamination. For example, where possible, the net opening should be protected from air exposure before and post sampling. Along with vessel speed and net opening area, GPS coordinates

should be recorded at the beginning and end of each tow to allow calculation of distance travelled, volume sampled, and estimate microplastic concentration ($V = \pi * r^2 * h$, where r = radius of the net and h = distance net towed). To ensure sampling provides an accurate representation of microplastics presence and distribution at the sea surface, at least 1 m^3 of water should be sampled – noting this will be dependent on the level of plastic pollution at the sampling site. Note that inferring volume solely based on distance travelled can lead to inaccurate estimations of the volume of water as water current flow is often not measured or accounted for. To achieve greater accuracy in volume estimation a flow meter can be attached to the bottom and/or centre of the net mouth. Replicates at each tow site should be conducted to report data variability. Where collection of replicates is not feasible (i.e., only one tow is conducted before conditions change), this should be clearly indicated, with the knowledge that no standard error or variance can be reported).

Following the sampling event, the entire net must be winched on board and rinsed from the outside using an on-board deck hose (either seawater or freshwater) to transfer trapped contents into the cod-end. Where feasible (e.g., logistically and following QA/QC), any large natural material captured should be removed using metal tweezers and, to dislodge any adhered microplastics, its surface should be rinsed liberally through the net into the cod-end container (the rinsed items can then be disposed of). Physical properties of the rinsed item should be documented. If the sample is dense and rich in organic material, this should be documented to guide the first step in the sample processing protocol, i.e., if chemical digestion is needed to remove smaller organic matter including biofilms (see Processing of microplastics section below). Large plastic debris items captured in the net can also be removed and rinsed through the net into the cod-end container, and stored separately for further analysis. The cod-end contents should then be transferred to a pre-cleaned bottle (e.g., 1-2 L), preserved and stored until analysis (e.g., fridge, 20% ethanol – see storage below). Additionally, the use of positive controls and blanks during sample collection is highly recommended (see Quality assurance and control section).

Before starting the next tow, a clean cod-end, or a used one that has been liberally rinsed with filtered water, should be attached to the net.

Submersible pump sampling

Submersible pumps are another alternative to survey water bodies and are most commonly deployed just below the surface or in deeper water (Rodrigues et al., 2018). Usually, these pumps are systems retrofitted to vessels (e.g., already existing seawater intake systems that are adapted to suit microplastic collection) or portable and able to be deployed via a winch system (Karlsson et al., 2020). While fixed systems have the advantage of minimal

handling and can sample along transects (e.g., while in transit or semi-continuously), these can only be used at one depth, and exclude surface microplastics (Schönlau et al., 2020). Portable systems, on the other hand, are suitable for depth profiling and can potentially be deployed from anywhere (i.e., not vessel dependent), but are not well suited to transect sampling. Portable pumps are, however, often expensive and may require skilled personnel to oversee their operation.

The likelihood of airborne contamination occurring while using submersible pumps depends on the pump design, and whether the pumped water is exposed to the air at any stage during collection. To prevent this, many submersible pump systems are designed so that specialised clean filters are only exposed to the air when being removed and replaced. However, some pumps might pump the water over an open sieve, and therefore these samples can be exposed to airborne contamination during collection. When samples are susceptible to airborne contamination during collection, blank controls should be used. These include vials containing ultrapure (e.g., Milli-Q) water that are exposed to air simultaneously with the exposure of samples; these are then passed through every procedural step. Regardless, the use of positive controls and blank controls during sample collection is highly recommended for submersible pump samplings (see Quality assurance and quality control).

The GPS coordinates of the sampling site should be recorded at the beginning of the activity. If a transect is being collected, GPS coordinates should also be recorded at the end of each sampling event to allow for reporting on distance travelled and calculation of the total volume filtered.

The volume of water sampled by submersible pumps is dependent on the system configuration and the depth at which it is deployed. Like for surface water sampling, at least 1 m^3 of water is recommended to be collected, allowing first and foremost for further vertical comparison. The use of a flow meter is advised where possible, however, if not available, the volume of water sampled can be calculated using the time the pump is operated (i.e., the sampling period) and the calibrated timeframe in which a given volume of water is pumped. Replicates at each sampling site should be completed to report data variability. Where the collection of replicates is not feasible (e.g., the pump is being used for semi-continuous surveys or technical issues have occurred), this should be recorded, noting that no standard error or variance can be reported.

Samples collected on filters should be immediately removed from the pump and stored in clean containers (e.g., labelled petri dish) to avoid exposure to air. Filters with samples can be stored cold (e.g., -20°C, fridge, on ice – see storage below). When samples are collected over sieves, sampled material must be transferred (i.e., backwashed) into

suitable clean containers (e.g., 150 mL vials) using ultrapure water or filtered seawater. These samples can then be preserved cold or in 20% ethanol (i.e., filtered ethanol is added to the sample). If more samples are to be collected via sieving, a new or cleaned sieve should be used.

Fixed submersible pumps are not always able to be thoroughly cleaned (nor visually inspected), especially vessel intake systems. For these pumps, it is important to flush the system well before the start of sampling to ensure any plastics trapped in the system do not cross-contaminate samples.

Bulk water bottling

Water quality sampling is traditionally undertaken with bulk water bottling (e.g., Niskin bottles attached to a Rosette sampler, or bottles directly) deployed in a specific location at a specific defined depth (Nan et al., 2020; Ogunola et al., 2023; Song et al., 2014). This same static sampling method is used to sample microplastics in water and across vertical gradients. When collecting surface water samples, the bottles should be deployed downwind or on the side of the vessel and ideally under calm weather conditions. Collection bottles must be appropriately cleaned before use (e.g., rinsed 3 times with ultrapure or filtered seawater and visually inspected). The configuration of the microplastic sampling system and bottle type can vary, so it is important to record the volume collected and time. The volume required is also dependent on the downstream analysis workflow. For example, if using pyrolysis-GC/MS to detect microplastics, 2 L is appropriate, whereas for other spectroscopy methods 10 L is advised. To limit the risk of airborne contamination, sampling bottles should remain sealed and only be opened and closed when underwater (Samandra et al., 2023). Additionally, the use of positive controls and blank controls during sample collection is highly recommended (see Quality assurance and quality control).

Preliminary processing of these samples is often conducted on the vessel as storage of large volumes is often not possible. The Niskin sample is generally filtered (e.g., over a stainless-steel mesh under vacuum) and the filters are stored in a sealed container (i.e., labelled glass petri-dish) until analysis. When filtering on the vessel is not possible, collected water should be transferred to a pre-cleaned bottle (e.g., 1-2 L), preserved (e.g., fridge, 20% ethanol – see storage below) and stored until analysis.

Storage

Following collection, and regardless of the collection method, all samples should be processed immediately or appropriately preserved until further processing. Although plastic items do not themselves require preservation (i.e., many are expected to persist in the environment for many hundreds of years), organic material is often also part of the

collected material. If samples are not preserved this organic matter can degrade and encourage the growth of microbes on the surface of the microplastics, which can impact their retrieval, identification and chemical analysis.

Sample preservation methods include low temperature (e.g., ice, -4°C fridge, -20°C freezers), or chemical treatments (e.g., ethanol, 3% hydrochloric acid), the latter with the caveat that only chemicals that do not impact plastics should be used (Pfeiffer & Fischer, 2020). Where possible, preliminary processing to clarify samples and enhance their long-term storage should be applied. For example, filtration can reduce volume, washing with ultrapure water (e.g., Milli-Q), <20% ethanol, or sonication (three cycles for five minutes each based on Kaiser et al., (2017)), can dislodge any biofilms attached to the microplastic surface, thereby enhancing buoyancy and contributing to more accurate and effective separation, identification and subsequent analyses.

In instances where immediate sample preservation or processing is not feasible, considerations need to be made for the influences that biological material can have on the retrieval and identification of microplastic particles.

Sample processing and analyses

After collection of the water samples, the microplastics need to be isolated for quantification and identification. Following good laboratory practice, a sample audit should be conducted, and all relevant environmental parameters and sample information entered into a project database or spreadsheet.

In this section, we briefly summarise the equipment and materials needed to process water samples. As well as briefly describe the steps to separate and quantify microplastics, physical and chemical characterisation and QA/QC.

Processing of microplastics (1 µm - 5 mm)

Equipment

Material

- Glass Petri dishes and beakers
- Metal forceps/tweezers
- Stainless steel filters
- Millipore gridded membrane (nitrocellulose filters)
- Metal sieve (sieve diameter and mesh aperture size need to be reported; the latter to understand the lower size limit)

- Density meter (for testing the density of the separation reagent)

Reagents

- Ultrapure water (e.g., Milli-Q)
- Preservation reagent (e.g., 10-20% ethanol, 70% ethanol)
- Density separation reagent (e.g., sodium chloride, sodium iodide or other outlined below)
- Chemical digestion reagents (e.g., 30% hydrogen peroxide, 10% potassium hydroxide or other outlined below)

All reagents and solutions should be filtered (<20 µm) before use, excluding the ultrapure water, which is already filtered.

Instruments

- Temperature controlled oven (optional)
- Orbital shaker (optional)
- Heated stirring plate (optional)
- Glass magnetic stirring rod (optional)
- Laminar flow (recommended)
- Filtration equipment (glass or stainless steel), including vacuum pump
- Stereomicroscope
- Polymer identification instrument (e.g., FTIR, µ-FTIR, ATR-FTIR, RAMAN, py-GC/MS)

Pre-treatment and procedures for microplastic separation

Water samples with low organic content

Water samples that visually appear clear can be fast-tracked through the isolation process to either density separation or filtration, with no chemical digestion needed. If preserved frozen, samples (still covered) should first be defrosted at room temperature. If preserved in a chemical solvent (i.e., 20% ethanol), care should be taken following the chemical solvent's Safety Data Sheet (e.g., regarding PPE and others).

Water samples with medium/high organic content

If the water sample contains phytoplankton, zooplankton or other organic matter that hinders the retrieval of microplastics, a clarification step is required prior to filtering. This clarification can be achieved using density separation (see below), but for samples with higher organic content, preliminary chemical digestion may be required (see below).

Chemical digestion

Chemical digestion can be applied as a first step to separate microplastics from seawater. There are a range of different chemicals that are appropriate, depending on research question, laboratory facilities and reagent availability (Table 2). The type of chemical digestion chosen is a compromise between efficiency, cost, and the known health and safety risks and potential for polymer degradation (Di Fiore et al., 2024; Miller et al., 2017; Pfeiffer and Fischer, 2020)(Table 2).

As an example, described below is a procedure that has been applied to facilitate the chemical digestion of a surface water samples with a high algae content. Potassium hydroxide (10% KOH) was added directly into the filtered seawater samples and then heated to a maximum of 40°C in a controlled oven for up to 72 hours. Where the digestion rate of the organic matter was slow, i.e., there was still organic material present after 72 hours, a second digestion method using hydrogen peroxide (H_2O_2 30% w/v) was applied. The sample was again agitated at 40°C on a temperature-controlled magnetic stirrer in a fume hood and the reaction was allowed to go to completion (follow Safety Data Sheet for all chemicals). Thereafter, samples went through density separation.

Table 2. Summary table of digestion reagents, their concentrations, advantages and limitations. Based on: Di Fiore et al. (2024) and Pfeiffer and Fischer (2020).

Digestion	Recommended concentration	Advantages	Limitations	Degradation of polymers?
Hydrogen peroxide (H_2O_2)	15-30%	Efficient, cheap	Corrosive, hazardous	Yes, at high concentrations
Hydrochloric acid (HCl)	20%	Efficient, cheap	Corrosive, hazardous, toxic, can degrade polymers	Yes
Nitric acid (HNO_3)	Weak	Efficient, cheap	Corrosive, hazardous, causes discolouration of plastics, can degrade some polymers	Yes
Sodium hydroxide (NaOH)	1 M	Efficient	Corrosive, hazardous, can degrade some polymers	Yes
Potassium hydroxide (KOH)	10%	Efficient	Corrosive, hazardous	Yes, at high concentrations
Enzymatic digestion		Safe, efficient	Expensive, time intense	Yes

Density separation

For large water samples density separation can be applied as a first step to separate microplastics from seawater or following chemical digestion. Concentrated water samples are resuspended in the brine solution of choice with less dense materials (e.g., microplastics) floating and becoming separated from denser materials (sediment, debris, others) present in the matrix. There are a range of different reagents that can be used for density separation. These differ in their density, and each has advantages or limitations associated with efficiency for microplastic recovery, varying levels of toxicity, and price range (Rani et al., 2023) (Table 3). Commonly used reagents include sodium chloride, sodium tungstate dihydrate, sodium bromide, sodium polytungstate, lithium metatungstate, zinc chloride, zinc bromide and sodium iodide. The effectiveness of reagents is polymer

dependent, e.g., higher density reagents are more effective at removing more dense polymers. The density of the final solution used must be reported, as it will dictate which plastic polymers can or cannot be retrieved with this methodology.

Note, samples that have been preserved (e.g., 20% ethanol) or chemically digested, may still contain some of the reagents and there is a risk that these will react with the density separation reagents, therefore, it is important to first neutralise the sample. The preserved or digested sample must be rinsed liberally with ultrapure water onto a filter or sieve before the density separation step. Always refer to the Safety Disposal Sheet of the chemical reagents for appropriate use and disposal.

To achieve density separation, the reagent should be added to the sample first, and then stirred (e.g., with a metal spoon or glass rod) and the solution allowed to settle. Stirring and settlement periods can vary based on sample complexity, and should be tailored with preliminary and spike recovery tests. Once a sample is completely stratified, the established supernatant should be poured over an appropriate filter (i.e., metal mesh, glass microfiber, silicon coated filter) using a filtration device (preferably metal or glass). The walls of the sample container should be rinsed liberally with ultrapure (e.g., Milli-Q) or filtered water to ensure all microplastic items are transferred. The filter should be stored in a labelled petri dish and covered with a glass lid. To identify microplastics, captured items should be observed under a microscope using any one of the microplastic quantification steps outlined below.

Table 3. Commonly used density separation reagents, highlighting their advantages and limitations.

Density separation reagent	Density of solution	Advantages	Limitations
Sodium chloride (NaCl)	1.2 g cm^{-3}	- Recovers low density polymers (e.g., PE, PS, PP, PMMA, PC) - Cost effective - Non-toxic - Readily available	- Less efficient at recovering high density polymers (e.g., PC, PU, PET, PVC, PTFE)
Sodium tungstate dihydrate ($H_4Na_2O_6W$)	1.4 g cm^{-3}	- Recovers low density polymers (e.g., PE, PS, PP, PMMA, PC) - Cost-effective - Non-toxic	- Less efficient at recovering high density polymers (e.g., PC, PU, PET, PVC, PTFE)
Sodium bromide (NaBr)	1.37 g cm^{-3}	- Recovers a wide range of polymers - Non-toxic	- Expensive
Lithium metatungstate ($Li_2O_{13}W_4^{-24}$)	1.62 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Unknown effect on polymers
Zinc chloride ($ZnCl_2$)	1.5 - 1.7 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Zinc bromide ($ZnBr_2$)	1.71 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Sodium iodide (NaI)	1.6 - 1.8 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Calcium chloride ($CaCl_2$)	1.5 - 3 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment

Potassium Iodide (KI)	1.7 g cm^{-3}	- Recovers a wide range of polymers - Non-toxic	- Expensive

Filtration

Water can be poured through the filter or sieve and gravity will drain the sample, or a vacuum pump can be used to accelerate the filtration and if there is a high particulate content. The configuration of the filter or sieve can vary, i.e., an individual filter or sieve can be used, or a tiered filtration unit (Schlawinsky et al., 2022). Either way, the smallest filter size must be recorded, as this will define the smallest microplastic sample that can be collected (e.g., if the sieve is 100 µm, microplastics smaller than 100 µm will not be accurately or consistently captured).

For samples preserved in a chemical solvent, the dilution of the preservation chemical should be such that there is <1% in the final washings. Crystallization and retention of inorganic salts in the sample solids can occur after filtering and may hinder both the physical and chemical analysis of microplastics. To prevent this, it is crucial to thoroughly and liberally rinse the filtrate with ultrapure water (e.g., Milli-Q).

Following filtering, filters with solids can be stored in labelled glass jars or covered petri dishes until microscope examination. The filtration unit should then be thoroughly rinsed with ultrapure water (e.g., MilliQ) and carefully inspected to ensure no residual solids remain and to prevent cross-contamination of the next sample.

Sediment

Pre-Survey Preparations

The methods for the collection of microplastics in marine, coastal, and riverine sediment can vary depending on the sediment composition (i.e., grain size, organic content) and environment (i.e., subtidal - always submerged; intertidal - the area between high and low tide; and supratidal - the area above the high tide line and only flooded occasionally during storms).

Sampling design

Before commencing field sediment sampling, it is necessary to formulate the research question(s) (see What are the goals of my research?). Different research questions may require different sampling designs and, likely, different sample collection methods. Furthermore, sampling designs should consider the post-survey requirements, including the time required to accurately process samples, the techniques to be used to analyse them and the units of reporting.

The next step is to define the spatial and temporal extent of the research question. Spatial studies can provide information on the presence of and changes in the distribution of microplastics across sites. Therefore, the sampling design must consider the number and spatial dispersion of the collection sites to answer the research question, site accessibility, the zone to be surveyed (i.e., intertidal vs supratidal), the site profile, geographical and oceanographic features, accumulation dynamics, the expanse of the area, and other environmental characteristics that may influence results. Temporal studies can provide information on changes in microplastic concentrations at a specific location over time, therefore the sampling design must also consider site accessibility across seasons and during/after significant weather events. For example, coastal environments are prone to significant shifts and fluxes in features (i.e., coastal erosion or submergence) due to rises in sea levels (i.e., king tides) and changes in wave action and currents from events such as flooding and cyclones.

Research conducted across spatial and/or temporal scales requires a stratified sampling design that enables an estimate of the true microplastic density (or type, weight, volume) at different scales or "strata" that represent clearly defined groups (Quinn and Keough, 2002). Samples should be collected randomly from each group. Stratified sampling should also be used when within-habitat variation is high, and it is not feasible to sample entire sites (CSIRO 2022). Refer to the Survey Design SOP for further details.

Sampling design and approach is dependent on study objective and the environment being sampled, e.g. quadrats or transects for intertidal habitats, grabs, quadrats, or cores for subtidal habitats (see descriptions below). Budget and logistical constraints also need to be considered as does the translation of methods for uptake by citizen science monitoring programs (which are now a key vehicle for conducting global surveys). Sampling approach can also define limitations on the size class of microplastics that can be analysed.

The number of samples collected will be determined by the research question as well as the budget, logistics and resources available; with the caveat that a high number of samples allows for greater accuracy (through power and replication) in the estimation of microplastic concentrations in the environment and will ensure greater statistical rigour. It is important to consider what would be an acceptable difference between the estimate and the true density or volume of microplastics (i.e., the effect size). Before commencing sample collection, a power analysis can be performed to assist in this decision. G*Power (Faul et al., 2007) or pwr package in R (Champley et al., 2020) are suitable for simple sampling designs and can determine the number of samples required to achieve the desired effect size.

There are two main approaches when examining transects for microplastic presence in subtidal, intertidal or beach environments (note this can include monitoring the entire transect, or quadrats along the transect, depending on the research question). The first is the parallel to shoreline, including the 'strandline' approach (which will record debris from the last high tide), where a set length of the beach is measured, and microplastic quantified (example in Figure 2, shows collection along the high tide line). The second is the perpendicular to shoreline approach, which records changes in microplastic density towards the tideline if/when the transect is surveyed in discrete sections. Both strandline and perpendicular methods are appropriate, and their use will depend on the research question. It is important to record details of environmental conditions, physical features at the site (e.g. sandy vs rocky beach, long beach vs small cove), and weather conditions (e.g., tides, storm surge, wind, wave action) (see environmental variables and the Reporting information section).

It is essential to record information on sampling effort as this can create variability in the results. This is particularly important with community-led beach surveys (e.g., citizen science monitoring programs), where there is variation in the number of people participating across time, how many hours are spent searching, level of competency, and area searched. These are important aspects that should be considered during sampling design and prior to commencing collection. Whilst other methods (e.g., grabs) are not

impacted by the number of surveyors, it is essential that the number of samples and replicates are clearly identified.

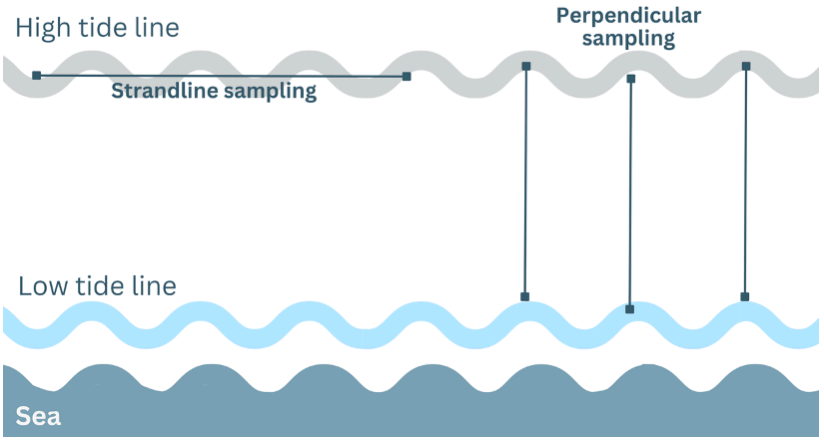


Figure 2. Sampling approaches for microplastic monitoring in intertidal environments.

Field Procedures

Materials and equipment for collection

Table 4. List of equipment and materials required for field sample collection in intertidal/supratidal and subtidal habitats. Asterisks* indicate items that are essential for microplastic sampling.

Intertidal and supratidal	Subtidal
Measuring tape*	Grab or coring device (e.g., box corer, van Veen grab, sediment corer) *
GPS start and end coordinates*	GPS start and end coordinates*
Quadrat*	Quadrat (for scuba diving survey)
Metal shovel or spoon*	Metal shovel or spoon
Depth gauge	Metal ruler
Stainless steel sieves or filters	Stainless steel sieves or filters
Glass petri dishes	Glass petri dishes
-	Syringe and suction tube*
Glass jars, aluminium tray or sterile press-and-seal plastic bags or paper envelopes (for dry sediment samples) *	Glass jars or zip lock plastic bags*
Labels*	Labels*
Field observation datasheet*	Field observation datasheet*
Pencils, permanent marker*	Pencils, permanent marker*
Camera	Camera
Glass filtering flask	Glass filtering flask

General principles

Ocean and coastal environments are dynamic and can be highly variable, so it is recommended to sample over time (e.g., seasonally) if feasible. Seasons may vary

depending on the region being sampled, in some areas of Australia there will typically be four seasons (summer, autumn, winter and spring) or two (wet and dry), but these may be further split based on local microclimates and different cultural group definitions. When sampling over time is not feasible, recording and reporting information on weather and other environmental conditions at the time of sample collection is important for contextual information (see Reporting section).

In the field during intertidal/supratidal, subtidal, and other environments, transects for perpendicular or strandline sampling (depending on the research question) should be measured for length. Additionally, GPS coordinates at both the beginning and end of the transect recorded. Photographs of the sampling transect should be taken. A randomised and stratified sampling design should be implemented, with a minimum of three replicate samples taken randomly along the transect. It is important to note, however, that the number of replications needed along the transect will depend on the microplastic load and the accumulation dynamics of the site. If there are large abundances of microplastic counted, three replications are needed, however, if there are low counts of microplastics, variations in the environmental conditions (e.g., variations in sediment type or grain size across transects), or other confounding factors, more replications are required. Likewise, sampling locations can be randomised (i.e., using a random number generator) along the transect. The GPS coordinates for each sampling location must be recorded.

Airborne and handling contamination can occur during field sampling, so appropriate quality assurance and quality control (QA/QC) procedures must be consistently applied throughout sample collection (see Quality assurance and control section). This includes the use of positive controls and blanks during sample collection.

Intertidal/supratidal

The collection methods employed in the intertidal and supratidal zones are similar. There are two main approaches for investigating microplastics in these two zones - standing stock assessments, and accumulation assessments. The first (standing stock assessment) involves collecting or counting the microplastic pieces which are already present on the beach (Fisner et al., 2017). This type of survey method gives a value about the microplastics at a given time in a given area. The latter approach (accumulation assessment) involves clearing the plastic away from the tideline initially, and then counting and/or collecting the microplastics that are carried by the waves (Galgani et al., 2013; GESAMP, 2019). This allows for information on the microplastic accumulation from the water.

There are a number of ways to count and/or collect microplastics, depending on the research objectives in intertidal and supratidal zones. Investigation of visible microplastics (1 mm - 5 mm) is usually done by counting the number of microplastics within a defined quadrat (e.g., 25 cm x 25 cm, 50 cm x 50 cm or other) often along a transect, with microplastics counted visually from standing height (estimated to be one metre directly above). This method does not require handling of the sediment, however, if desired, the top layer (1-5 cm) of sediment from the quadrat can be collected and sieved (i.e., using a 1 mm aperture metal sieve) to capture microplastics for subsequent chemical analysis.

If microscopic microplastics (1 µm - 1 mm) are to be counted the sediment will need to be collected and transported for further processing. The sediment can be collected in a number of ways and is dependent on the site accessibility and storage and transport capacity. Recommended approaches include, collecting the top layer of sediment (between 1 - 5 cm) within the entire quadrat using a metal shovel and depth gauge (Piperagkas et al., 2019; Wessel et al., 2016). The size and depth of the sample collected should be recorded, along with sample mass (can be measured retrospectively). Alternatively, a quadrat can be placed and randomised replicate samples within the quadrat collected (e.g., using a small corer, vial, or shovel). A minimum of three replicates are recommended to allow for variance to be calculated within each quadrat.

Subtidal

There are generally five approaches used to collect subtidal sediment – grab samplers, core samplers, dredge samplers, or quadrats via SCUBA and Remotely Operated Vehicles (ROVs).

The depth and sediment type to be collected, and the mode of collection, will depend on the research question. Where possible, retrospective sampling design needs to be optimised to collection mode, i.e., whether the mode of collection is manual or automated, and to the site.

If using grab, core, dredge or ROV samplers, consideration must be given to the grain size, the sampling regime (i.e., top 5 cm of sediment surface vs subsurface cores), the effort required to sample, and the potential for incidental resuspension of microplastics, which can result in an underestimation of microplastic concentrations. In some cases, collections by grab can be further subsampled using a corer.

Other environments

The methods outlined above can be tailored and transferred to additional land and aquatic habitats, both freshwater, estuarine, and marine. For land habitats, (e.g., sand dunes beyond the supratidal zone, mudflats, riverbanks, mangroves) the intertidal/supratidal methods should be followed. While for aquatic habitats (e.g., river sediment, estuaries, coral reefs) the subtidal methods are likely more appropriate (Skalska et al., 2020). When tailoring these sample collection methods, the sampling design should always be considered, and the modified method validated. Likewise, the accessibility and features of the site may dictate the ability to perform transects, so changes may be required. Overall, providing detailed information on sample collection is an essential step to ensuring broad study comparisons (see Reporting information).

Storage

Following collection, and regardless of the collection method, all samples should be processed immediately or preserved until post-survey processing procedures can be completed (see Quality assurance and quality control section). In specific circumstances samples may need to be frozen (e.g., for cores, and to minimise disturbance of layers) or dried to reduce the likelihood of microbial and algal growth (Adomat et al., 2022). Storage containers and vials should only be opened whilst transferring the sample to minimise any extraneous airborne contamination.

Where possible, preliminary processing either in the field or as soon as possible after collection should be applied to enhance their long-term storage. For example, elutriation and/or density separation methods can quickly reduce sediment mass, but note both require optimising to the sediment type.

Although plastic items do not themselves require preservation (i.e., many are expected to persist in the sediment for many hundreds of years) organic matter is often also part of the collected material. If samples are not preserved, this organic matter can impact the processing, retrieval, identification and chemical analysis of microplastics. Where needed, samples should be stored cool at -4°C (limited time) or frozen at -20°C or -70°C (for cores). In instances where immediate sample preservation or processing is not feasible, considerations need to be made for the influences that biological organisms can have on the retrieval and identification of microplastic particles.

Sample processing and analyses

After the collection of sediment samples, the microplastics need to be isolated for

quantification and identification. Following good laboratory practice, a sample audit should be conducted, and all relevant environmental parameters and sample information entered into a project database or spreadsheet.

This section briefly summarises the equipment and materials needed to process sediment samples and describes the steps to separate and quantify microplastics, including their physical and chemical characterisation and QA/AC considerations. These procedures can be undertaken either in the field or laboratory, depending on environment, safety considerations, and available facilities in the field.

Processing of visible microplastics (1 mm - 5 mm)

Equipment

Material

- Glass Petri dishes and beakers
- Metal forceps/tweezers
- Metal sieves [sieve diameter and mesh aperture sizes need to be reported; the latter to understand lower size limit (e.g., 1 mm)]

Reagents

- Density separation solutions (e.g., Sodium chloride, Potassium iodide, Zinc chloride). See Table 5 below for details
- Chemical digestion reagents (e.g., 30% hydrogen peroxide, 10% potassium hydroxide)
- Ultrapure water (e.g., Milli-Q) or filtered water

Instruments

- Stereomicroscope (recommended, but will be essential for microplastics <1 mm)
- Polymer identification instruments (e.g., FTIR, u-FTIR, ATR-FTIR, RAMAN, py-GC/MS, LDIR) (desirable for polymer type)

Procedure

Sediment should be sieved through an appropriately sized metal sieve (e.g., 1 mm) and retained microplastics sorted, counted, and identified (see Microplastic characterisation and quantification). Larger natural debris (i.e., algae, shell, sticks, biota) should be removed with forceps or tweezers and liberally rinsed with ultrapure water (e.g., Milli-Q) or filtered water over the sieve to remove any microplastics adhering to their surface. It is recommended a microscope be used where feasible.

Sediment type, range in grain size, and levels of organic matter can influence the ease of sieving and the downstream separation and identification of microplastic. In the case of finer sediments (i.e., silt, mud, clay), density separation or pre-treatments (i.e., chemical digestion) may assist in separating the microplastics from the sediment and/or organic matter. See below for more details on density separation and chemical digestion. While recommended, it may not always be feasible to include these steps, e.g., in field surveys associated with citizen science monitoring programs. In these and other circumstances, it is critical to accurately report collection information and the minimum size range of plastics that can be captured and visually detected (see Reporting section).

Processing of microscopic microplastics (1 µm - 5 mm)

Equipment

Materials

- Glass Petri dishes and beakers
- Metal forceps/tweezers
- Stainless steel filters
- Metal sieves (varied sized, sieve diameter and mesh aperture size need to be reported; the latter to understand lower size limit (e.g., 1 µm))
- Acid-resistant plastic box with lid

Reagents

- 30% w/v hydrogen peroxide (H_2O_2) - for organic matter removal
- Fenton's reagent (H_2O_2 and FeSO4)
- Density separation solutions (e.g., Sodium chloride, Potassium iodide, Zinc chloride. See Table 2 below for details on appropriateness of different reagents).
- Ultrapure water (e.g., Milli-Q) or filtered water

All reagents and solutions should be filtered (<20 µm) prior to use, excluding the ultrapure water, which is already filtered.

Instruments

- Laminar flow cabinet (optional)
- Filtration equipment, including vacuum pump
- Stereomicroscope (essential for microplastics <1 µm)
- Oven or freeze drier
- Polymer identification instruments (e.g., FTIR, µ-FTIR, ATR-FTIR, RAMAN, py-GC/MS, LDIR)

Procedures

Sediment drying

Depending on the processing method and units of reporting, sediment samples may need to be dried, either in a freeze drier (loosely covered), or in a low heat oven (covered). The time and temperature required to dry the sample will vary depending on the sediment type, grain size, organic load, sample mass, and mode of drying. Caution should be taken when heating sediment samples as plastics may be impacted at temperatures above 40°C. Following drying and depending on the research question, the sample can be homogenised by sieving with a mechanical mixer or hand mixing (with care to ensure no fragmentation of plastic occurs). For larger samples, a subsample of a specific weight (e.g., 100 grams) can be collected (i.e., using a metal corer), ideally this should be done in triplicate to ensure sample representativeness.

Chemical digestion

A pre-treatment to remove organic matter from organic-rich sediments is recommended. This aids in the clarification of the sample and means that the subsequent application of density separation solutions is likely to be more effective, it also allows for the use and re-use of more expensive density separation solutions which are often needed to isolate higher density microplastics.

There are a number of methods that can be used to digest sediment bound organic matter including 30% w/v H_2O_2 solution or Fenton's reagent (H_2O_2 and FeSO4; the ferric ions act as a catalyst). However, care should be taken when considering degradation of polymers when certain reagents are left for periods of time or temperatures (Di Fiore et al., 2024; Miller et al., 2017; Pfeiffer and Fischer, 2020). When using H_2O_2 and Fenton's reagent, the solution should be added to the sediment, mixed with a metal spoon or glass rod for one minute and left covered (e.g., watch glass) for up to 18 hours (depending on the amount of organic matter). To increase the efficiency of this reaction, the solution can be heated to 40 - 50°C to enhance the digestion process (note that temperatures above 40°C may impact and degrade particular polymers more than others). Based on time (i.e., 18 hours) or clarity (i.e., no visible organic matter remaining) or temperature (the reaction is exothermic and will cool once digestion is complete), the reaction should be quenched by washing thoroughly with ultrapure (e.g., Milli-Q) or filtered water (i.e., formation of carbon dioxide bubbles stops). Fenton's reagent can be challenging with the efficiency of the digestion dependent on the pH of the sample, therefore prior to use further reading on the use of Fenton's reagent is recommended (e.g., Tagg et al., 2017).

Elutriation

Where organic content is not high, an elutriation pre-treatment to process larger masses of marine and coastal sediments is recommended. Elutriation is a process designed to separate lighter particles from heavier ones, using an upward stream of gas or liquid (Claessens et al., 2013; Hengstmann et al., 2018; Kedzierski et al., 2016; Zhu, 2015), thereby rapidly reducing the initial volume of the sediment sample and concentrating finer solids and debris for subsequent density separation. To separate microplastics from larger sediment samples (i.e., >500 g), samples can be transferred into a tall column (e.g., ~1 m, and preferably of glass or stainless steel) connected to a freshwater source (via a tap at the base of the column). The water flow is controlled through a valve handle and should be monitored by a flowmeter. To avoid the introduction of potential contaminants from the plumbing, the water must first pass through a filtering system (e.g., 1 µm cartridge filter)before entering the base of the column. The water then passes through a 26-µm mesh that ensures a homogeneous distribution of water flow inside the column. When the tap is open, the sand becomes fluidised and the lighter particles are carried to the top of the column. As the water flows over the outlet any floating solid material is collected on a metal sieve with the desired µm aperture (e.g., 10 µm). The optimal flowthrough is dependent on the sediment granulometry and should be determined by spiking experiments (e.g., for a 1 m column, a 1400 L per hour water flow for 3 minutes is optimal for beach sand). The loaded sieves should be covered (e.g., with aluminium foil) and transferred to the laboratory for further processing via density separation.

Density separation

To separate microplastics from sediments, samples are resuspended in a density separation solution (choice determined by the type of plastics likely to be present), where less dense materials (e.g., microplastics) will float and separate from denser materials (sediment, debris, others) present in the matrix. There are a range of different reagents that can be used for density separation. These differ in their density, and each has advantages or limitations associated with efficiency for microplastic recovery,
varying levels of toxicity, and price ranges (Rani et al., 2023)(Table 5). These include sodium chloride, sodium tungstate dihydrate, sodium bromide, sodium polytungstate, lithium metatungstate, zinc chloride, zinc bromide, sodium iodide (Crutchett and Bornt, 2024). Higher density reagents are more effective at removing higher density polymers and are often employed when studying sediments, particularly those from intertidal and subtidal environments where heavier plastics sink and settle into the benthic zone. The density of the final solution used must be reported, as it will dictate which plastic polymers can or cannot be retrieved with this methodology.

To extract the microplastics using density separation reagents, the sediment should be transferred into a previously decontaminated glass beaker and the density separation reagent added. The sediment and density separation solution should be gently stirred with a metal spoon or glass rod and allowed to settle. Using a filtration kit (preferably glass or stainless steel), pour the supernatant over an appropriate filter (i.e., metal mesh, glass microfiber, silicon coated filter). The walls of the glass beaker should be rinsed liberally with ultrapure (e.g., Milli-Q) or filtered water to ensure all microplastic items are transferred to the filter. The loaded filter should be stored in a labelled Petri dish and covered with a glass lid. To identify microplastics, captured items should be observed under a microscope.

Table 5. Commonly used density separation reagents, highlighting their advantages and limitations.

Density separation reagent	Density	Advantages	Limitations
Sodium chloride (NaCl)	1.2 g cm^{-3}	- Recovers low density polymers (e.g., PE, PS, PP, PMMA, PC) - Cost effective - Non-toxic - Readily available	- Less efficient at recovering high density polymers (e.g., PC, PU, PET, PVC, PTFE)
Sodium tungstate dihydrate ($H_4Na_2O_6W$)	1.4 g cm^{-3}	- Recovers low density polymers (e.g., PE, PS, PP, PMMA, PC) - Cost-effective - Non-toxic -	- Less efficient at recovering high density polymers (e.g., PC, PU, PET, PVC, PTFE)
Sodium bromide (NaBr)	1.37 g cm^{-3}	- Recovers a wide range of polymers - Non-toxic	- Expensive
Lithium metatungstate ($Li_2O_{13}W_4^{-24}$)	1.62 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Unknown effect on polymers
Zinc chloride ($ZnCl_2$)	1.5 - 1.7 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Zinc bromide ($ZnBr_2$)	1.71 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Sodium iodide (NaI)	1.6 - 1.8 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Calcium chloride ($CaCl_2$)	1.5 - 3 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment

Potassium Iodide (KI)	1.7 g cm^{-3}	- Recovers a wide range of polymers - Non-toxic	- Expensive

Biota

IMPORTANT NOTE: *Prior to conducting any work on marine or coastal biota in relation to plastic and microplastic contamination, you must confirm what the permit, ethics, biosecurity, and work health safety requirements are for each specific species of interest. On confirming what is required, no work on marine and coastal organisms should begin until all relevant authorities have granted approval and formal documentation has been attained. Please assess both state and federal requirements.*

Pre-Survey Preparations

The methods to acquire samples (i.e., capture, collection) of aquatic biota for microplastic assessment may vary according to the environment (e.g., pelagic vs benthic) and status (e.g., live vs dead) of the target species. The information in this section is organised into distinct sampling approaches tailored to major categories of marine and coastal biota.

Monitoring microplastic in marine and coastal biota

Analysis of microplastics in aquatic biota is generally conducted under one of the following overarching themes:

- To monitor microplastic presence in the aquatic environment. Bioindicator species, such as bivalves (e.g., mussels, oysters) or plankton, can accumulate microplastics and reflect the presence of contaminants in the ecosystem. Analysing these species over time and space, allows assessing the extent and variations in the presence of microplastics in coastal and marine ecosystems to be assessed.

- To investigate microplastic occurrence, accumulation and impacts on biota and across food webs. This involves examining, for instance, the ingestion, accumulation, and potential biological effects of microplastics on organisms, including bivalves, fish, marine mammals, and seabirds, among others. These investigations generally aim to assess the risks posed by microplastics to species and to provide valuable insights into the broader ecological implications of microplastics. This includes biota sampled opportunistically, such as carcasses of deceased individuals, and investigation of plastic presence in the gastrointestinal tract (GIT).

At the time of writing, microplastics have been detected in 1,565 species of wild animals (Santos et al., 2021) from aquatic and terrestrial ecosystems, and this number continues to grow as more species are assessed. However, approaches to monitor microplastics in

different biota differ widely due to the inherent differences in size, morphology, digestion, the way the species interact with plastic, the species accessibility for research, animal ethics considerations and permissions, as well as the cultural norms/attitudes surrounding research on different taxa. This is especially the case when it comes to lethal sampling designs, which are sometimes utilised to achieve non (or less) biased sampling for plastic ingestion research, given that many species reduce or alter their feeding behaviour before death. Non-lethal and opportunistic sampling designs can also yield valuable information on microplastic exposure without the need to sacrifice living animals, although these designs are also prone to sampling biases, which will be explored later.

Generally, it is more common to conduct targeted lethal microplastic exposure baseline and monitoring sampling designs on smaller organisms such as smaller invertebrates (non-cephalopods), although there are exceptions. Lethal sampling designs typically involve the collection or harvest of live animals for the explicit purpose of scientific research. These animals are sacrificed, and plastics contained within them are separated from the organic matter through chemical digestion of the entire animal, post necropsy, or from GIT contents (i.e., ingesta and digesta), and then microplastics quantified. Non-lethal sampling designs, on the other hand, are usually employed when investigating larger organisms (non-fish vertebrates). These often involve either the collection of scats/faeces or regurgitated material from living animals, which can be collected from the environment, or by capturing the animal to procure the sample (i.e., via gastric lavage, or as part of routine health assessments).

Opportunistic sampling designs are common for larger vertebrates, especially those that are either logistically difficult to access (for example rare or dispersive species) or where ethical and cultural values would curtail permissions to disturb the animal or its habitat for microplastic research (e.g., threatened and otherwise protected species). Opportunistic sampling design often involves the collection of carcasses of animals that have died of natural/incidental causes, been euthanised for veterinary/welfare purposes, or killed for a purpose that is removed from research goals such as aquaculture, wild harvest or by-catch of harvest for human consumption.

While it is not feasible here to include a sampling design for all the species that are investigated for microplastic research, general principles of sampling design, microplastic separation and plastic characterisation and reporting apply. Included here are the main general principles and an overview of approaches for different taxa.

Species selection

For vertebrates, most of the microplastic research around the world has used opportunistic samples, typically necropsy of animals that have died due to a variety of causes. Opportunistic samples are typically sourced from beach-cast individuals, euthanised for welfare purposes, or by-catch (and deceased) in fishing and other commercial activities. However, the number of animals that can be sourced this way in Australia is generally low, not only because of the low numbers of animals dying, but also a lack of organised, accessible centralised systems for reporting and collecting carcasses (e.g., centralised beach monitoring networks, though some jurisdictions have local, site-level or informal citizen-science monitoring networks), which is the case even if the scope of sampling effort is Australia-wide. For example, fisheries by-catch is only reported in specific fisheries, or when there are observers onboard, and carcasses of by-catch animals are typically discarded at sea rather than collected for scientific purposes. For cetaceans, it is unlikely that more than ten individuals are likely to be procured each year unless there is a mass mortality event, such as a mass stranding. It is important to note that permits are still required to investigate these animal carcasses. Therefore, for sampling wild organisms, it is important to plan ahead for the logistic reality of potential low sample numbers, difficulty achieving scientifically robust replication, stratification, and other best practice sampling goals.

The following criteria should be considered when selecting species to investigate and/or monitor:

- Naturally occurring species with a high abundance,
- Species for which robust sampling and processing methods are already established,
- Ease of sampling and laboratory processing,
- Species already commonly used as bioindicators in other areas of research, i.e., marine pollution and conservation monitoring programs,
- Species of ecological, socio-economic and cultural importance,
- Species with a broad coverage of ecological niches, and feeding types,
- Species that do not require ethics for sampling (e.g., those not considered sentient),
- Or, species for which structured/standardised sampling already occurs, and for which the addition of microplastic sampling is possible.

As an example, bivalves are a popular choice given that they fulfil most of these criteria (Ding et al., 2021; Li et al., 2019):

- they are not currently considered to be sentient,
- do not require ethics,
- are common in intertidal zones and coastal locations globally,

- are relatively easy to collect from the wild,
- can be sourced from commercial growers or raised in laboratory settings,
- have high economic and ecological value, and
- there is a plethora of literature describing validated methods and their successful implementation in biomonitoring programs.

Other species that have been used as indicator species include small fish (specifically, by sampling their GIT contents) and sediment-dwelling organisms (e.g., worms). A review of plastic bioindicator literature for the North Pacific identified a suite of 12 candidate bioindicator species covering a variety of ecosystem components and a wide range of plastic size classes, including two bivalves, three fish and two sea turtles species that are also present in Australia (Savoca et al., 2022).

Note on Listed marine wildlife (EPBC Act, Marine and Migratory or Threatened)

Marine mammals, marine turtles, sea snakes, and several species of seabirds and elasmobranchs are listed as Matters of National Environmental Significance (EPBC Act). This means they are subject to many levels of protection and there is a high barrier for entry for disturbance of these taxa for research generally. Due to this, lethal sampling methods for protected species are very unlikely to be permitted by any authority in Australia. Non-lethal sampling methods are difficult to apply to vertebrate marine wildlife that do not routinely regurgitate, and whose faeces are passed in the seawater, making scat collection logistically difficult.

Sampling design

General principles

The first thing that must be considered before commencing field sampling is what question(s) are you trying to answer (see What are the specific goals of my research?). Different research questions may require different sampling designs and most likely different sample collection methods. Refer to the Survey Design SOP for further details. Sampling designs should also ensure post-survey requirements are adequately considered, as well as the time required to accurately process microplastic samples from biota. Overall, sampling design principles are similar irrespective of whether you are seeking to use biota as a proxy to monitor for the presence of microplastic in the environment or whether you are specifically concerned with monitoring microplastic impacts on specific biota.

Sampling frequency is also dependent on the research question and species being investigated. If variations over time or between seasons are under question, then multiple sampling efforts across seasons or time are required. Ideally, replication is required within each spatial or temporal unit of interest. If simply trying to quantify whether microplastic is present, then a single targeted sampling effort may be appropriate. Notwithstanding, following these guidelines will ensure the comparability of microplastic data from biota.

Pros and cons of designed vs opportunistic sampling

Designed sampling approaches

Biota can be sampled through structured designed sampling approaches - for example, the targeted harvest of a species through a well-planned sampling design.

Examples of designed sampling approaches include:

- Bivalves placed in sea cages at predetermined distances from a river mouth to monitor the reach of microplastic outflow.
- Microplastic loads in polychaete worms sampled in the intertidal zone every kilometre along a coastline of interest.
- Microplastic load in every 50th sardine harvested from wild-caught sardine fished commercially for human consumption.
- Microplastic frequency and loads in scats collected weekly over 12 months across a suite of pinniped colonies.

Strengths

- Careful control over sampling design means that robust sampling principles such as stratification, randomisation, replication and adequate sampling power can be built in at the design phase,
- Allows for standardisation and comparability,
- Often larger sample sizes, i.e., can assess multiple individuals of a population at any given sampling time,
- Can assess how representative the sample is of the wider population,
- Suitable for long-term monitoring to understand trends in microplastic concentrations, and
- Ideal for differentiating any observed trends.

Weaknesses

- Often inappropriate for studies involving whole-organism examination, such as the necropsy of vertebrates (except for fish harvested for human consumption) due to the ethical and legislative hurdles of collecting/harming/disturbing these species for a research purpose,

- Not suited to the investigation of threatened or endangered species in the event biota is sacrificed, and
- Sampling to achieve adequate replication likely to be resource intensive.

Opportunistic sampling approaches:

Biota can be sampled through structured or non-structured opportunistic sampling approaches, for example, the collection of carcasses as they become available.

Examples of opportunistic sampling approaches include:

- Microplastic frequency of occurrence, load and characteristics across a suite of by-catch species available through commercial fishing,
- Microplastic frequency of occurrence, load and characteristics in pilot whales sampled during a mass stranding event,
- Microplastic frequency of occurrence, load and characteristics sampled in carcasses of seabirds and sea turtles that died in care at a marine animal rescue centre, and
- Microplastic frequency and loads in scats collected during a single trip to a pinniped colony.

Strengths

- Opportunistic access to rare species and species that move that are difficult or not possible to sample live in the wild, for example, beach-cast animals, stranding events and by-catch of cetaceans, sea turtles and seabirds,
- Opportunistic access to species that ethical permissions would not be granted for targeted disturbance or harvest, for example, threatened species and those covered by legislation for example, protected by the EPBC Act 1999,
- Can provide preliminary data on previously under investigated species, and
- Is often cheaper.

Weaknesses

- Prone to sampling biases such as single populations, discrete geographic areas, and other local conditions/contexts that may not be appropriate to generalise,
- Prone to biases affecting the behaviour of the animal before collection, for example, sick animals that become beach-cast may not be feeding normally before death,
- Often smaller sample sizes, i.e., can be a single individual, and
- Difficult to assess how representative the sample is of the wider population and to determine variability.

Field Procedures

Materials and equipment for sample collection

The vast range in size (e.g., from invertebrates to whales), life cycles, anatomy and environment (pelagic vs benthic) of aquatic biota species means that the tissue or type of sample collected varies greatly (Table 6). Likewise, biota can be sampled directly from the environment or from fisheries and aquaculture. Therefore, specific consideration of taxa traits and their habitats is needed to determine the most appropriate collection method – some of these include trawl nets, cages, line, or hand collection by snorkel or SCUBA (Zantis et al., 2021) (Table 7).

When collecting any sample from marine and coastal biota (from plankton to whales, and benthic to pelagic) or faeces, scat and regurgitate, whether in the field or captivity, it is essential to implement measures to reduce contamination during the collection (e.g., reducing risk of contamination from collection gear; rinsing of external bodies to remove adhered environmental microplastics) (Lusher et al., 2017). Where possible, organisms should be individually weighed, and size measurements taken prior to the dissection and/or extraction of any tissues. If specific dissected tissues are being analysed for microplastic content, these should also be weighed. Samples should be stored in clean, plastic-free containers (preferably in glass) in clean areas, and processed in a clean laboratory environment (see the Quality Assurance and Quality Control (QAQC) section below for more details).

To correctly represent a population, an appropriate sample size that takes into account the research unit (e.g., species, food web, feeding type) needs to be selected. Power analysis is strongly recommended when planning collections and experimental designs. It is important to consider what would be an appropriate effect size. Before commencing sample collection, a power analysis can be performed to assist in this decision. G*Power (Faul et al, 2007) or pwr package in R (Champley *et al.* 2020) are suitable for simple sampling designs and can determine the number of samples required to achieve the desired effect size. Sample sizes can be smaller if appropriately justified (e.g., organism availability, statistical power analysis, and monitoring constraints), e.g., see Lavers et al. (2021) for a power analysis calculated to sample microplastics in birds.

Table 6. The type of sample that can be collected for analysis of microplastics by organism type. This table is a reference only. Literature reviews and targeted preliminary and further research are required to confirm that the sampling method is appropriate for the organism of interest and suited to the downstream analyses. PPE - Personal Protective Equipment. The ✓in the table indicates the method(s) that can be used for the aforementioned categories.

Sample Type	Cetaceans & Dugongs	Pinnipeds	Marine Turtles & Marine Reptiles	Marine Birds	Fish & Elasmobranchs	Bivalves	Larger Crustaceans	Pelagic Invertebrates	Benthic Invertebrates
Whole organism					✓	✓	✓	✓	✓
Trawl					✓			✓	
Faeces	✓	✓	✓	✓	✓				
Opportunistic necropsy	✓	✓	✓	✓	✓				
Opportunistic regurgitation				✓					

Table 7. Equipment that is required for field sampling of different aquatic biota groups. This table is a general guide as each sampling strategy will have variations in equipment and sampling requirements.

Sampling Method	Cetaceans & Dugongs	Pinnipeds	Marine Turtles & Marine Reptiles	Marine Birds	Fish & Elasmobranchs	Bivalves	Larger Crustaceans	Pelagic Invertebrates	Benthic Invertebrates
Permits	✓	✓	✓	✓	✓		✓	*	
Ethics	✓	✓	✓	✓	✓		*	*	
PPE	✓	✓	✓	✓	✓	✓	✓	✓	✓
Trawl net					✓			✓	
Fishing gear					✓	✓	✓	✓	✓
Quadrant & relevant equipment						✓			✓
Coring equipment									✓
Netting				✓					
Sample collection and storage equipment i.e. Glass jars (appropriately cleaned)	✓	✓	✓	✓	✓	✓	✓	✓	✓
General field equipment (Datasheets, labels,	✓	✓	✓	✓	✓	✓	✓	✓	✓

pencils)									
Camera and GPS	✓	✓	✓	✓	✓	✓	✓	✓	✓

*Cephalopods require ethics approval and may need a permit in some jurisdictions.

Common cross-taxa processing approaches and opportunities for harmonisation

Across the different taxa, there are several common approaches to sample processing and analysis. For general information on standard microplastic separation protocols, see the detailed post-survey sections below, which include details on visual inspection, separation of microplastics from organic matter using chemical/enzymatic digestion and/or density separation, and visual and chemical identification of microplastics. Many of these protocols are suitable for numerous species and tissue types and offer compelling starting points and significant opportunities to increase harmonisation in sample collection, processing, and reporting. These include:

- Necropsy procedures for large animals (cetaceans, sea lions and large sea turtles)
- Necropsy procedures for medium sized animals (sea birds, small sea turtles, large to medium fish)
- Chemical digestion of ingesta and digesta
- Chemical/enzymatic digestion of soft tissues (GI tracts of fish and large crustaceans, soft body of bivalves, soft-bodied pelagic and benthic invertebrates)
- Chemical digestion of chitinous species (small crustaceans, hard-bodied pelagic and benthic inverts)
- Chemical/enzymatic digestion of whole animal (small fish, fish larvae and sponges)
- Collection and digestion of faeces and scats
- Microscopy protocols
- Polymer identification

However, given the diversity of taxa and sample types (e.g., tissues, faeces, scat, regurgitate) that are now being investigated for microplastics presence and quantification, specific considerations must be given to taxa- and sample-specific protocols for downstream sample processing. Furthermore, in many cases, published protocols are not available and there is a need to establish and validate methods before use (Santana et al., 2022).

Taxa-specific collection and processing strategies

Cetaceans and dugongs

Sampling cetaceans and dugongs

Notwithstanding their iconic status and protection in many jurisdictions globally, cetaceans and dugongs are not ideal candidates to monitor or research microplastic because they are large and logistically difficult to work with.

Most microplastic sampling studies of cetaceans come from the collection and processing of faeces and gut contents from smaller live wild species (i.e., dolphins), and through the

necropsy of by-catch, beach stranding and other cetacean carcasses (Lanyon, 2010). It should be noted that many cetacean species move, which means that the plastic data collected may not accurately represent the location of capture/sampling.

Separating plastic from organic matter

For cetaceans, necropsy is the primary method of separating plastic from organic matter, specifically, via post-mortem examination of the gastrointestinal tract (GIT). There are numerous cetacean post-mortem assessment protocols worldwide (Ijsseldijk et al., 2019; Mazzariol and Centelleghe 2017; Plön et al., 2015). For Australia, please refer to the Australian Government's standardised protocols (Australian Government, 2006).

One of the challenges for studying plastics in cetaceans, especially larger species, is that when cetaceans are necropsied, often there are many other multidisciplinary samples sought for pathology and other purposes (i.e., pathological evaluation, microbiological and virological analyses, algal biotoxin detection, diet and parasitological investigations), that are collected from the carcass, though standard protocols vary by country. Given the size of cetaceans and the large volume of their GIT contents compared to other organisms, it is common practice to collect plastics of the visible size range (> 1 mm), and uncommon to separate plastics of the microscopic size range. However, when cetacean GIT contents are examined for microplastics <1mm, they are found (e.g., Nelms et al., 2019a). Sieving of GIT contents through a series of sieves of decreasing mesh size, down to the desired minimum size range, is the standardised methodological approach (Corazzola et al., 2021) to investigate GIT contents of marine mammals, and allows for the simultaneous collection of samples for different disciplines, including plastics and microplastics. It also supports respective analyses and comparisons of multipurpose results.

For dugongs, faeces are commonly collected and processed for diet and health studies (Lanyon, 2010). There are no specific standard protocols for separating plastic from the organic matter in dugong faeces but chemical digestion and sieving and/or microscopic analysis are recommended (as detailed in post survey section below) (see also (Nelms et al., 2019b; Ortega-Borchardt et al., 2023).

There are no current standard protocols for GIT sample collection from dugong carcasses or GIT contents from lavaging. However, the general principles for cetacean sampling can be applied to dugongs. The Department of Foreign Affairs and Trade has a dugong necropsy protocol that must be followed (Eros et al., 2007). Following the removal of the GIT, if separate samples for different purposes are to be collected, including for quantification of (micro)plastics, the digesta should be treated following Corazzola et al. (2021).

Pinnipeds

Sampling pinnipeds

Most microplastic sampling studies of pinnipeds come from the collection and processing of scats, which are easily accessible on land at haul out sites, and through the necropsy of by-caught and other deceased pinnipeds.

Separating plastic from organic matter

Pinniped scats are widely collected and processed for diet and health studies, with plastic items separated from organic matter using a variety of methods; similar collection protocols can also be applied to microplastic assessments. Scats can be examined for either or both visible size range plastics and microscopic microplastics.

Though there are no standard protocols for separating plastic specifically from the organic matter in pinniped scats, chemical digestion and sieving and/or microscopic analysis are the recommended approaches (see post survey section below). Additionally, a range of studies have methods that would be appropriate to follow (e.g., Nelms et al. 2019b; Ortega-Borchardt et al. 2023)

Like cetaceans, for pinnipeds, the GIT is often removed from the carcass (sealed at both ends) to examine for plastics, and (micro)plastics can be separated from organic matter via the same sieving method used for cetaceans, following Corazzola et al. (2021).

Marine turtles and other marine reptiles

Sampling marine turtles

Study of microplastics in sea turtles relies primarily on the necropsy of deceased, by-catch, and beach cast animals and the removal of the GIT from mouth to cloaca. It is difficult to obtain scat in the environment or to sample faeces from live wild marine turtles, though faeces can be collected from animals kept in captivity, for example in rescue and rehabilitation facilities. Marine turtles do not regurgitate, so stomach lavage is the only method available to sample live wild turtle GIT contents.

Separating plastic from organic matter

It is common to find macro- and meso-sized plastics during sea turtle necropsy, and these should be reported, following standard protocols such as (Provencher et al., 2019a). The methods for separating (micro)plastic from organic matter vary, depending if the aim is to quantify plastic visible to the naked eye only (more common for marine turtle research;

items are hand-picked from the flayed GIT) or both visible size range and microscopic microplastics (less common in sea turtle plastic ingestion research; items are digested as per Caron et al. (2018). There are many other protocols available for microplastic separation from turtle GIT samples (Caron et al., 2016; Caron et al., 2018), many being similar to that of other larger taxa discussed above.

Marine birds

Sampling marine birds

There are multiple methods to investigate microplastic ingestion by marine birds. Organisms are collected either as a beach-wreck (often opportunistic), by-catch (semi opportunistic), or have died in care (car strike or post fledging, semi opportunistic) or those that are harvested or culled for non-research purposes (e.g., indigenous harvest, culling at airports, euthanasia for other illnesses). Necropsy and removal of the GIT is the main approach for dead seabirds.

For live birds, the collection of pellets (bolus) naturally regurgitated by some species may be possible, and for other species, other regurgitations (such as induced by birds' defensive behaviour or water-offloading technique) can be used. Scats can also be collected and inspected for microplastic presence, though this is only suitable for some species depending on the viscosity and ease of collection of the scat (Nelms et al., 2019b).

Separating plastic from organic matter

The choice of method to separate plastic from sampled marine birds will be dependent on the sample type being investigated, i.e., tissues from necropsy, pellets (boluses), other regurgitations and scats (Provencher et al., 2019b). The method will also depend on whether the observer is quantifying visible-sized microplastics only (more common for seabirds) or visible and microscopic microplastics (while this approach is less common at present, it is gradually becoming more common). For visible size range plastic, necropsy of seabirds can involve examination of just the upper GIT (proventriculus and gizzard/ventriculus) or the entire GIT from oesophagus to cloaca, followed by the visual inspection and/or sieving of GIT contents, and chemical digestion, if needed. For pellets (boluses), typically research just focuses on visible size-range microplastics, which are obtained by dissection of the pellet either by eye or under a microscope. For other regurgitates (e.g., lavage/water offloading), research typically focuses on visible size-range microplastics, which are easily retrieved from the lavage/offloaded water sample by eye; analysis of microplastics requires microscopic analysis. For scats, research focusing on microscopic microplastics is more common (few birds pass visible size range

microplastics); scat is generally processed using chemical digestion, followed by density separation (Keys et al., 2023) (see post survey procedures below).

Fish

Sampling fish

Designed and opportunistic sampling of fish harvested for human consumption are popular methods to sample for microplastic assessment. Due to the widespread harvest of a large variety of fish species across the world, fish are among the easiest organisms to sample for microplastic research. There are numerous protocols to capture fish for microplastic research (Markic et al., 2019; Wootton et al., 2021). Fish can also be opportunistically sampled as by-catch in fishing targeting other species. The GIT and gills are the most commonly collected tissues for microplastic assessment.

Separating plastic from organic matter

For smaller fish, chemical digestion of the entire animal is common. For medium to large sized fish, the most common methods involve necropsy of the GIT, and depending on the GIT size, a choice of processing the entire GIT for medium species, or analysing anatomical sections of the GIT or the ingesta/digesta for larger fish species using chemical digestion methods. Due to the popularity of fish as a species to sample and concern for human health through secondary ingestion of microplastic when eating fish, there is a wide variety of literature and protocols for the separation of plastic from organic matter in fish (Dawson et al., 2020; Dehaut et al., 2016; Karami et al., 2017; Karlsson et al., 2017; Lusher et al., 2017; Wootton et al., 2021) (see post survey procedures below).

Bivalves

Sampling bivalves

Bivalves are among the most popular taxa for microplastic monitoring and research, using both designed and opportunistic sampling approaches, with several countries running national microplastic monitoring programs using bivalves. For example, the Republic of Korea performs national monitoring using Pacific oyster (*Crassostrea gigas*), blue mussel (*Mytilus edulis*), and Manila clam (*Ruditapes philippinarum)* (Cho et al., 2021). The use of bivalves for microplastic monitoring is growing in popularity. National biomonitoring programmes like the long-running 'Mussel Watch' initiative by NOAA in the United States of America, and by others in Europe use local mussel species to assess environmental contamination, and can incorporate microplastic assessments (National Oceanic and Atmospheric Administration, 2020; Provenza et al., 2022).

There are a variety of sampling approaches used to sample microplastics from bivalves. Sampling is typically repeated over time given that environmental microplastics may vary across four seasons (Summer-Autumn-Winter-Spring) or rainy (wet/monsoon) and dry seasons in tropical regions. Given that Australia spans latitudinally from the tropics to temperate zone, seasonality should also be factored into bivalve microplastic monitoring efforts in Australia.

Bivalves (e.g., mussels, oysters, pipis) can be sourced from wild populations or commercial growers (e.g., aquaculture). Methods of collection include manual removal by twisting (mussels), hammer and chisel to remove from substrate (mussels, oysters) and trowel/spade and sediment pumps (pipis). Bivalves should be euthanised in an ice-slurry and transferred to -20°C freezer for storage. For the explicit purpose of biomonitoring, cages of selected bivalve species can be deployed in select locations (e.g., following the Mussel Watch approach). Five families of bivalve have been typically monitored. In order of popularity at the time of publishing, these are: mussels, oysters, clams, scallops, and cockles (Ding et al., 2022).

Separating plastic from organic matter

Given the small size of most bivalves, to quantify plastics within organisms, chemical or enzymatic digestion of the entire soft tissue (not usually including the shell) of the organism is the most popular (Catarino et al., 2017). Given the small size of microplastics ingested by these organisms, microplastics that are visible to the naked eye are rare, and microscopy and polymer identification are necessary (see post survey procedures). Other research objectives, include investigating rates of ingestion and depuration, and the consequences of microplastic exposure in live animals held in tanks in the laboratory (Hamm et al., 2022; Yap et al., 2020).

Large crustaceans (e.g., crabs, lobsters, prawns)

Sampling crustaceans

Crustaceans, many being detritus feeders, are becoming increasingly popular taxa for microplastic research, using both designed and opportunistic sampling approaches, especially for species that are commercially harvested for human consumption. Crustaceans are collected usually by traps and nets, or by hand via snorkelling or SCUBA .

Separating plastic from organic matter

Depending on the size of the crustacean, removal and chemical digestion of the GIT only

(for larger crustaceans) or chemical digestion of the whole organism (for smaller organisms) are prevalent (Lusher et al., 2017). The crustacean exoskeleton and chitinous lining, however, represents a significant challenge for the digestion of the whole animal, and standard chemical and enzymatic methods are not always suitable (Li et al., 2022). Given the small size of microplastics ingested by these organisms, microplastics that are visible to the naked eye are rare, and microscopy and polymer identification are necessary (Dehaut et al., 2016).

Pelagic invertebrates (including plankton)

Sampling pelagic invertebrates

Sampling pelagic invertebrates is commonly performed simultaneously with sampling plastics in seawater, either at the sea surface or in subsurface waters. Given the relatively passive or small-scale movement of many pelagic invertebrates with the ocean currents and their large geographic ranges, they serve as valuable subjects for research aiming to establish connections between plastic concentrations in waters (exposure) and in organisms (load). Sampling pelagic invertebrates is often conducted using net-based trawls (typically with a mesh size of 335 mm, but this can vary depending on the target species), such as manta trawl nets, bongo nets, hydra bios nets, and multi-depth samplers, but also with niskin bottles. These methods are used to effectively collect plastics at the sea surface as well (see marine and coastal water section), and consequently, the abundance of plankton and plastics in water samples is often measured concurrently.

Separating environmental plastic from organic matter

The method used to separate environmental microplastics from the plankton is dependent on the abundance of organic material present in the sample. Samples with low plankton (and other organic material) abundance can be manually sorted using Bogorov chambers and dissection scope, or (with rinsing) over sieves with aperture size suited to the target species. Samples with high plankton abundance will require complete chemical digestion.

Separating ingested plastic from the organism

Given the small size of most pelagic invertebrates, quantifying plastics within (i.e., ingested by) these organisms necessitates chemical digestion of the entire specimen or community of organisms (Egger et al., 2022). Chemical digestions require a sequential process involving the density separation of microplastics from the pelagic community and/or visual inspection of the external surfaces of the sampled community. This precaution ensures that the plastics detected post-digestion originated from within the organisms, as opposed to being present in the environment or adhered to the external surfaces of the organisms.

Given the small size of microplastics ingested by these organisms, microplastics that are visible to the naked eye are rare, and microscopy and polymer identification are necessary (see post survey procedures).

Benthic invertebrates

Sampling benthic invertebrates

Sampling benthic invertebrates can be, and is often, done at the same time as sampling sediment (e.g., via grabs, see grab field manual). As many benthic invertebrates have limited geographic ranges, they are popular in research seeking to link plastic concentrations in sediments (exposure) and in invertebrates (load). Benthic invertebrates can also be collected by hand, and via snorkelling or SCUBA. Larger benthic invertebrates (i.e., sea cucumber, gastropods, tunicates) can be further dissected and relevant tissues removed for microplastics processing (Santana et al., 2022).

Separating plastic from organic matter

Many benthic invertebrates are small in size (i.e., coral polyps, marine worms), therefore, to quantify plastics within these organisms, chemical digestion of the entire organism followed by polymer confirmation are recommended (Dehaut et al., 2016; Lusher et al., 2017; Santana et al., 2022). Dissected tissues, including the whole GIT, from larger invertebrates (i.e., sea stars, sea cucumber, gastropods) are routinely digested whole (Santana et al., 2022).

Sample processing and analyses

Storage

To ensure sample integrity and to reduce the possibility of extraneous contamination during storage, samples should be placed in appropriately sized, clean storage containers (preferably glass, where possible).

For whole organisms, individuals or populations (i.e., plankton) can be immediately frozen following collection (ideally at -20ºC), thereby effectively stopping all body processes including gut clearance. Where possible, whole organisms should be stored flat to minimise the likelihood of internal fluids leaking or draining out. Other preservation methods may be preferred, aligned with research questions (e.g., associated with impacts of microplastic on biota), although to align contamination with endpoints, understanding of the impacts of the different preservation methods on microplastic retrieval or decomposition may be required (see Quality assurance and quality control section).

If analysing the gastrointestinal tract (GIT) for the presence of microplastics in either whole organisms or dissected GIT, it is important to reduce, or preferably stop, gut clearance prior to sample processing and analysis.

For dissected GIT, the two ends of the GIT should be sealed (e.g., tied closed) and stored flat (to prevent draining from one GIT compartment to others) and immediately frozen (ideally at -20°C). For larger GIT, the GIT may be cut into smaller compartments (according to the animal's anatomy); again, the two ends of each compartment should be sealed and stored flat and immediately frozen.

Sample processing

Once the biota samples have been collected, the microplastics need to be separated for quantification and identification. Following good laboratory practice, a sample audit should be conducted, and all relevant environmental parameters and sample information entered into a project database or spreadsheet. This is particularly important if working with species that are collected under ethics and/or permits, as there will be periodic reporting requirements to meet.

A brief summary of methods used to process biotic samples and a description of the equipment and materials needed to process biotic samples is provided.

Materials

- Glass petri dishes and beakers
- Metal forceps/tweezers
- Metal mesh or glass microfiber filter
- Metal sieve (sieve diameter and mesh aperture size need to be reported; the latter to understand lower size limit (e.g., 1 mm))
- Separating funnel

Reagents

- Digestion reagent, commonly H_2O_2 or KOH (see Table 8, and respective section below). Concentration will depend on the type of organic matter, mass of the biotic samples being digested, and other factors such as whether a shaker or heat bath are being utilised.
- Enzymes for enzymatic digestion

- Ultrapure water (e.g., Milli-Q)

All reagents and solutions should be filtered (< 20 µm) prior to use, excluding the ultrapure water, which is already filtered.

Equipment

- Temperature controlled oven
- Filtration equipment, including vacuum pump
- Stereomicroscope
- Heat bath and/or shaker
- Polymer identification instruments (e.g., FTIR, µ-FTIR, ATR-FTIR, RAMAN, py-GC/MS)

Chemical and enzymatic digestion procedures for microplastic separation

Although the literature is being updated frequently as microplastic research advances and matures, it is highly recommended that, where feasible and before starting sample processing, the literature for the specific tissue type being examined be thoroughly reviewed and tissue digestion methods carefully assessed for suitability. Also, because different digestion reagents can impact polymers differently, this should be taken into account when selecting the digestion method, the caveat being that no single method is best or safe for all polymer types.

A digestion step is strongly recommended when investigating microscopic microplastics in biotic samples, to ensure all microplastics can be released from the organic matrix and to achieve a suitable level of clarification to easily identify microplastics under the microscope. There are a range of different chemical and enzymatic reagents that are suited to tissue digestion, the choice dependant on research question, laboratory facilities, availability of reagents and, most importantly, tissue type (Table 8). Ultimately, the chemical digestion method used is a compromise between efficiency, cost, and the known risks and possibilities of polymer degradation (Table 8).

Three 'general approach' chemical digestion methods are recommended: 15% hydrogen peroxide, 10% potassium hydroxide under 40°C heat, and enzymatic digestion. Recommendations are broadly based on the balance between the advantages they impart, the limitations they introduce, and the impact on polymers (Table 8).

Table 8: Summary table of digestion reagents, their concentrations, advantages and limitations. Based on: Di Fiore et al. (2024) and Pfeiffer and Fischer (2020).

Digestion	Suggested concentration	Advantages	Limitations	Degradation of polymers?
Hydrogen peroxide (H_2O_2)	15-30%	Efficient, cheap	Corrosive	Yes, at high concentrations
Hydrochloric acid (HCl)	20%	Efficient, cheap	Can degrade polymers, corrosive, toxic	Yes
Nitric acid (HNO_3)	Weak	Efficient, cheap	Corrosive, discolouration of plastics, degradation of some polymers	Yes
Sodium hydroxide (NaOH)	1M	Efficient	Corrosive, can cause degradation of some polymers	Yes
Potassium hydroxide (KOH)	10%	Efficient	Corrosive	Yes, at high concentrations
Enzymatic digestion		Safe, efficient	Expensive, time intense	No

Air

Pre-Survey Preparations

The methods required for the collection of microplastics from the air are the same for all microplastic sizes and environments?.

Sampling design

Before commencing field sampling, it is necessary to formulate the research question(s) (see What are the goals of my research?). Different research questions may require different sampling designs and, likely, different sampling methods. Sampling designs should also consider post-survey requirements, including the techniques to be used to analyse them and the units of reporting.

The main issues of interest when addressing the presence of microplastics in the air are:

- Where, what or how much microplastic is there?
- Are there variations in microplastic in space or time?
- What are the sources of microplastic?

Sampling design and approach are dependent on the study objective and the environment being sampled. There are two main approaches when sampling the air for microplastics, namely active and passive monitoring. Active monitoring involves drawing in air through a collection system to capture microplastics. This is typically done using sampling devices that allow for controlled and continuous airflow and collection of airborne particles. Active monitoring provides real-time or near-real-time data on microplastic concentrations and can be useful for monitoring specific sources or events, such as industrial emissions or urban areas with high microplastic pollution. It is also suitable for studying the dynamics of microplastics in the atmosphere. Active sampling is often set up to collect air at the height of the human mouth, so can measure the potential exposure of humans, and therefore assists in assessing human health risks upon inhalation of microplastics. Passive monitoring involves leaving a collection substrate or device in a specific location to capture airborne microplastic deposition over a specific period of time. These substrates can then be analysed in a laboratory to determine the concentration and types of microplastics present. Passive monitoring is useful for understanding long-term trends and assessing microplastic presence in specific environments. Passive sampling is often more applicable for sampling remote areas, as there is no need for access to power.

Sampling frequency also depends on the research question. If variations over time or

between seasons are under question, then multiple sampling efforts across seasons or over time are required. In addition, replication is required within each spatial or temporal unit. Further, research conducted across spatial and/or temporal scales requires a stratified sampling design that enables an estimate of the true microplastic density (or type, weight, volume) at different scales or "strata" representing clearly defined groups (Quinn and Keough, 2002). Samples should be collected randomly from each group. Stratified sampling should also be used when within-habitat variation is high, and it is not feasible to sample entire sites (CSIRO 2022).

Despite this, if simply trying to quantify whether microplastic is present, then a single sampling effort may be appropriate. Notwithstanding, following these guidelines will allow new data that is collected down the track to be compared.

The number of samples collected will be determined by the research question as well as the budget, logistics and resources available; with the caveat that a high number of samples allows for greater accuracy (through power and replication) in the estimation of microplastic concentrations in the environment and will ensure greater statistical rigour. It is important to consider what would be an appropriate effect size (i.e. the acceptable difference between the estimate and the true density or volume of microplastics in the air). Before commencing sample collection, a power analysis can be performed to assist in this decision. G*Power (Faul et al., 2007) or pwr package in R (Champley et al., 2020) are suitable for simple sampling designs and can determine the number of samples required to achieve the desired effect size.

Field Procedures

Materials and equipment for collection

The materials and equipment required for collection of air samples depends on the type of monitoring occurring, that being either active or passive.

Active monitoring

- Air sampling device with vacuum pump and airflow meter (Figure 3)
- Source of electricity
- Metal forceps
- Metal mesh filter
- Glass Petri dish
- GPS
- PPE (avoid polyester and other plastic fibres)

- Pencils, datasheet, labels, permanent markers

Passive monitoring

- Glass collection device or filter holder
- Metal mesh filter
- Glass fiber filter
- Glass Petri dish
- Metal forceps
- GPS
- PPE (avoid polyester and other plastic fibres)
- Pencils, datasheet, labels, permanent marker

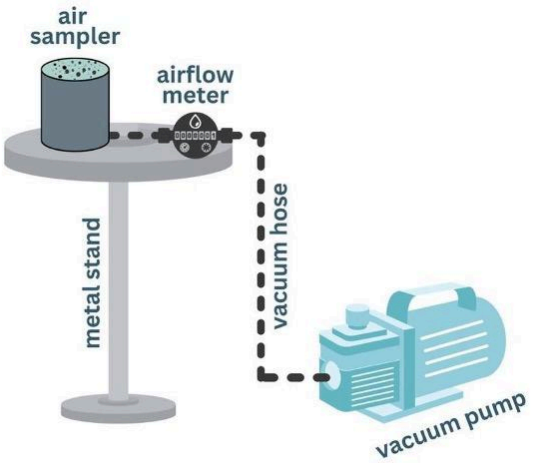


Figure 3. Diagram of the set-up of an active monitoring air sampling device.

Active monitoring

For both outdoor and indoor sampling, the sampling device should be placed at a specific, pre-defined height depending on the objective of the study (Perera et al., 2022). If the aim of the study is to test human exposure, it is recommended the device is set between 1.2 m (child standing height, or mouth height of a sitting adult) and 1.6 m. The flow rate must be consistent, and recorded. The recommended flow rate is 100 L/min, which is most commonly used. However, this rate can vary depending on the type of the sampling device used and the capacity of the machine.

When sampling indoors, it is recommended that less than 10% of the total indoor volume is

collected for the sample. To allow for appropriate replication, three independent samples should be collected at each location being tested, but consider study design and power analysis, as described above. Appropriate Quality Assurance and Quality Control procedures must be followed, including the use of blanks (See QAQC section).

Passive monitoring

For passive sampling, it is important to examine the quantity of airborne microplastics in both wet and dry atmospheric deposition to gauge the overall presence of microplastics in the environment. The wet collection sampler could include a cleaned open beaker with a glass funnel (Azari et al., 2023). For dry sampling, an open glass petri dish, or a glass funnel attached to a beaker would be appropriate.

For passive monitoring, the sampling device is placed in a specific predefined location in either an indoor or outdoor environment. To allow for appropriate replication, three independent samples should be collected at each location being tested, but consider study design and power analysis, as described above. The time over which the sample is exposed will depend on the research question (Chen et al., 2020; Knobloch et al., 2021).

Storage

Following collection, the mesh filter should be placed in a labelled glass Petri dish, and stored until analysis. This can be stored at room temperature if no biological material is included. If biological material is included, it should be refrigerated at 4°C until further analysis (Dong et al., 2021; Huang et al., 2021).

Sample processing and analyses

Once the air samples have been collected, the microplastics need to be isolated for quantification and identification. Following good laboratory practice, a sample audit should be conducted and all relevant environmental parameters and sample information should be entered into a project database or spreadsheet.

In this section, we briefly summarise the equipment and materials needed to process air samples, as well as briefly describe the steps to separate and quantify microplastics, their physical and chemical characteristics, and quality assurance and quality control.

The following digestion and density separation procedures may not be required if the samples are collected from relatively clean environments, which is commonly the case with active sampling. However, if samples are collected from polluted areas (e.g., industrial sites, near highways), further sample processing, including separating organic material, and density separation may be required. These are outlined below.

Equipment

Materials

- Glass Petri dishes and beakers
- Metal forceps/tweezers
- Stainless steel metal filters
- Metal sieve (varied sized, sieve diameter and mesh aperture need to be reported to understand lower size limit (e.g., 20 µm))
- Acid-resistant plastic box with lid
- Aluminium tray

Reagents

- 30% hydrogen peroxide (H_2O_2) (w/v) - for organic matter removal
- Density separation reagent (e.g., sodium chloride, sodium iodide or other chemicals outlined below in Table 9)
- Ultrapure (e.g., Milli-Q) water

All reagents and solutions should be filtered (<20 µm) prior to use, excluding the ultrapure water, which is already filtered.

Instruments

- Filtration equipment, including vacuum pump
- Stereomicroscope

Pre-treatment and procedures for microplastic separation

Organic matter removal

A pre-treatment to remove organic material from organic-rich samples is recommended. This helps subsequent processing steps, and means that the subsequent application of density separation solutions is likely to be more effective, it also allows for the use and re-use of more expensive density separation solutions which are often needed to isolate higher density microplastics. First, the sampled material should be transferred to a glass beaker. The H_2O_2 solution (30% w/v) should be added, mixed with a metal spoon or glass rod for one minute and left in a fume hood until all organic matter is digested (i.e., no

organic matter remaining). A reaction will occur degrading the organic matter, and once complete (formation of carbon dioxide bubbles stops) the reaction should be quenched by washing thoroughly with ultrapure water (e.g., Milli Q).

Density separation

Before the addition of density separation chemicals, samples must be thoroughly washed with ultrapure water to ensure there is no reaction between H_2O_2 and the chemicals used for density separation. Ultrapure water can be filtered first, prior to adding the density separation solution.

Air samples should be resuspended in the density separation solution of choice, where the less dense materials (e.g., microplastics) will float and separate from the denser materials (sediment, debris, others) in matrix. There are a range of different reagents that can be used for density separation. These differ in their density, and each has advantages or limitations associated with efficiency for microplastic recovery, varying levels of toxicity, and price ranges (Table 9). These include sodium chloride, sodium tungstate dihydrate, sodium bromide, sodium polytungstate, lithium metatungstate, zinc chloride, zinc bromide, sodium iodide. Different reagents work better for different densities of polymers, with higher density reagents more effective at removing denser polymers. The density of the final solution used must be reported, as it will dictate which plastic polymers can or cannot be retrieved with this methodology.

To extract the microplastics using density separation reagents, first, combine the liquid and the density separation reagent in a previously decontaminated glass beaker. Stir the solution with a metal spoon or glass rod and allow it to settle. Using a filtration kit, filter the supernatant using the chosen filtration device (i.e., metal mesh, glass microfiber, silicon coated filter). After filtering all the liquid contents of your jar, rinse all of the walls of the container to ensure all microplastic items are on the filter. Store the metal filter paper in a labelled cover Petri dish until microscope examination.

Table 9. Summary table of density separation reagents and their advantages and limitations. Post-survey Procedures

Density separation reagent	Density	Advantages	Limitations
Sodium chloride (NaCl)	1.2 g cm^{-3}	- Recovers low density polymers (e.g., PE, PS, PP, PMMA, PC) - Cost effective - Non-toxic - Readily available	- Less efficient at recovering high density polymers (e.g., PC, PU, PET, PVC, PTFE)
Sodium tungstate dihydrate ($Na_2WO_4.2H_2O_2$)	1.4 g cm^{-3}	- Recovers low density polymers (e.g., PE, PS, PP, PMMA, PC) - Cost-effective - Non-toxic	- Less efficient at recovering high density polymers (e.g., PC, PU, PET, PVC, PTFE)
Sodium bromide (NaBr)	1.37 g cm^{-3}	- Recovers a wide range of polymers - Non-toxic	- Expensive
Lithium metatungstate ($Li_2O_{13}W_4^{-24}$)	1.62 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Unknown effect on polymers
Zinc chloride ($ZnCl_2$)	1.5 - 1.7 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Zinc bromide ($ZnBr_2$)	1.71 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Sodium iodide (NaI)	1.6 - 1.8 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment
Calcium chloride ($CaCl_2$)	1.5 - 3 g cm^{-3}	- Recovers a wide range of polymers	- Expensive - Toxic to the environment

Potassium iodide (KI)	1.7 g cm^{-3}	- Recovers a wide range of polymers - Non-toxic	- Expensive

Microplastics quantification and characterisation

Microplastic characterisation is usually conducted at three levels (Figure 4), all of which provide key defining information on the microplastic. If the sample contains larger meso and macro plastics, these can often be readily identified visually without isolation, although polymer type still requires chemical confirmation. Similarly, if the experimental design requires that large microplastics (1 to 5 mm) are to be counted, as pieces are visible to the naked eye, the procedures applied at each level are less intensive – although the use of a binocular lens or similar is highly recommended. If small microplastics (1 µm to 1 mm) are also being quantified, a more thorough microscopic procedure is essential. The workflow for processing microplastic samples needs to be tailored to the research question, considering the nature of the sample at the time of collection (i.e., relatively clear vs organic rich), collection method, preservation method, available equipment, level of expertise and finally how the data is to be reported.

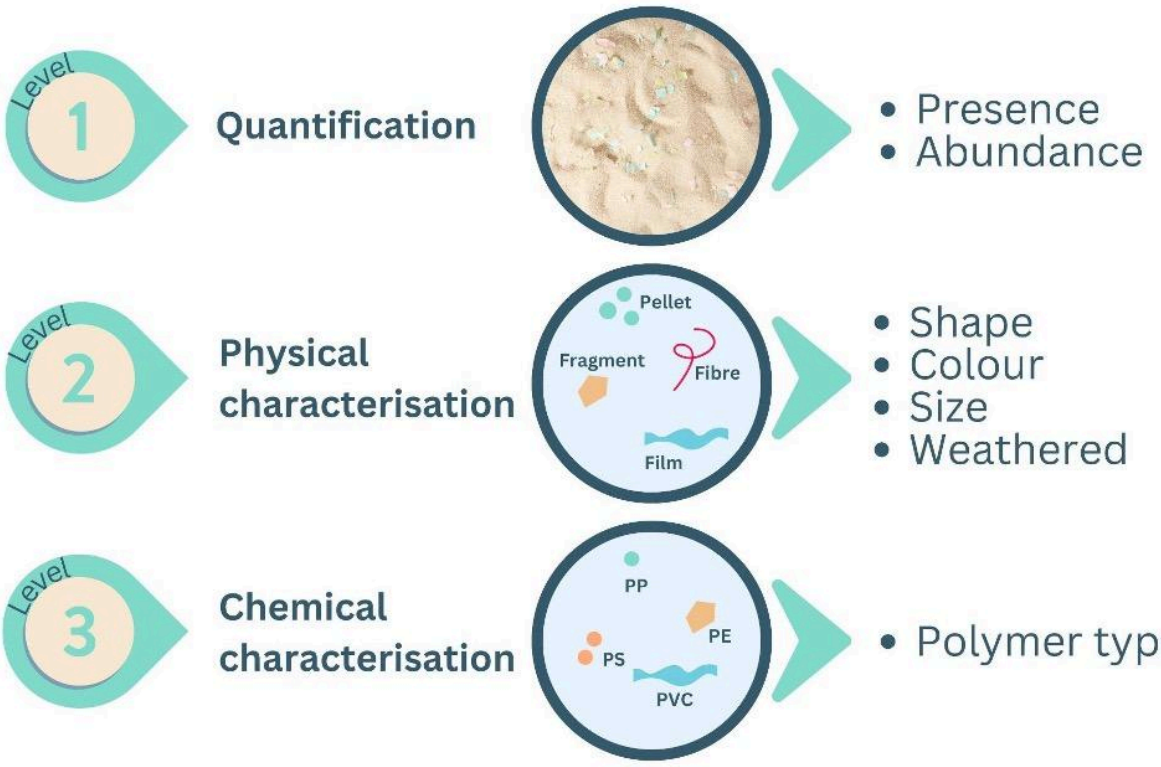


Figure 4. The three levels of microplastic characterisation and identification and the type of data produced. Figure adapted from Lynch et al 2023

Microplastics quantification

Visible microplastics (1 mm - 5 mm)

Quantifying visible microplastics does not necessarily require a microscope and they can often be counted using the naked eye, or weighed. Where further morphological information is required (i.e., texture, surface uniformity), a microscope is recommended. To count microplastics in the visible category the filter or sieve can be investigated by using the naked eye.

Microscopic microplastics (1 µm – 1 mm)

There are several ways to quantify microscopic microplastics (i.e., not discernible to the naked eye) once they have been isolated using methods described above (e.g., filter, sieve). Some approaches require manual counting under a microscope, while others rely on microphotography and specific software for semi-automated counting. Table 10 highlights a number of these methods and their advantages and limitations.

Table 10. Table of the different quantification methods for microplastics including their advantages and limitations.

Microplastic quantification method	Advantages	Limitations
Manual counting methods		
Gridded method	- Cost-effective - Straight-forward process	- Time intensive - Requires experienced operators - Observer bias
Fluorescent dyes (e.g., Nile Red)	- Cost-effective - User friendly	- Added processing steps and chemical considerations - Does not bind to all polymers - Dyes biological material (false positive)
Automated counting methods		
Image analysis software (e.g., Image J, CellSense image analysis)	- Highly accurate - Saves time - Automated - Removes human error	- Requires setup and calibration - Requires specific software - Requires high resolution microphotographs

Gridded method

Under a microscope, the gridded method involves examining and counting the microplastics in each square of a real or virtual grid (Brandt et al., 2021). If the microplastics are too numerous a subset could be counted in each square of two diagonals throughout the filter (see Figure 5 below as an example). However, it should be noted that this latter method may not always be representative of the sample.

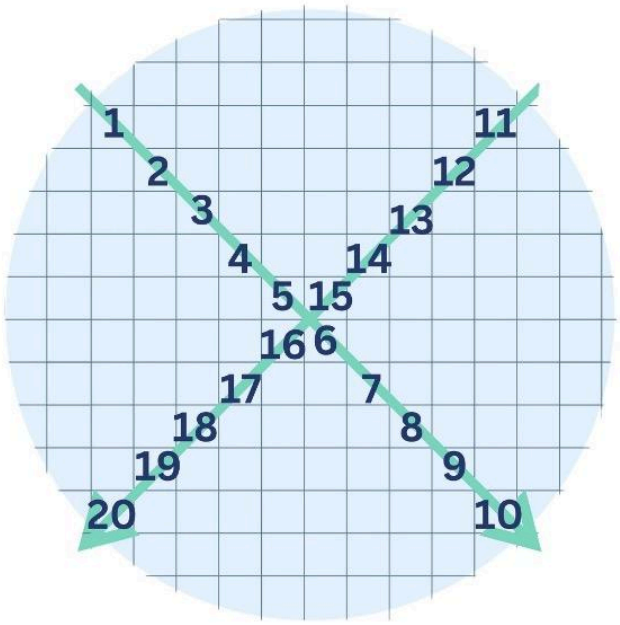


Figure 5. An example of the counting technique that could be used to quantify a subset of microplastics.

Fluorescent Dyes

Fluorescent dyes can be chosen based on their properties, including compatibility with the microplastics of interest, stability, and fluorescence characteristics. Nile Red is the most commonly used dye, however, Rhodamine B, Acridine Orange, Propidium Iodide and others can also be used. It is important to note that the choice of fluorescent dye depends on the research objectives, the specific polymers of interest, and the available instrumentation for detection. The dye is mixed with the environmental sample, processed following the post-survey procedures above, and examined using a fluorescence microscope. When illuminated with a specific wavelength of light (excitation wavelength), the fluorescent dye attached to the microplastics absorbs the light energy and re-emits it at a longer wavelength (emission wavelength), producing visible fluorescence, and highlighting the microplastics.

Image analysis software

Software such as ImageJ (Fiji), a free Java-based image processing program (U.S. NIH, MD, USA https://imagej.nih.gov/ij), CellSense, Saturna Imaging System (https://oceandiagnostics.com/microplastics-imaging-technology) and others enable semi-automated counting of microplastics. The data can be automatically exported from these software packages into a spreadsheet.

Once counted, the microplastics should be categorised primarily by type (fibre, fragment, film, foam, bead, etc) and colour, with other visual factors that support the identification of microplastics recorded, i.e., structure homogeneity, absence of cell structure or glossy surface (see Physical characterisation). All items categorised as putative microplastics should be chemically assessed to confirm their identity (see Chemical characterisation). If there are too many items, chemical assessment of a subset should be undertaken, documenting how the subset was selected and its representativeness determined. For microplastics treated with fluorescent dye, it is also important to identify the chemical signature of the dye, as this needs to be accounted for in later chemical analysis.

Physical characterisation

Accurate physical characterisation lies at the heart of understanding the nature and impact of microplastics in environmental samples. This section discusses the methodologies and considerations essential for characterising microplastic contamination based on their physical attributes. Plastic characterisation provides valuable insights into the size distributions, types, and other key physical properties of microplastic. Accurately reporting this information can allow researchers to further investigate the complexities of microplastic contamination and investigate trends. The methodologies outlined herein are designed to enhance the accuracy and consistency of microplastic analysis, contributing to a more comprehensive understanding of their presence and potential effects in various environmental matrices.

Reporting of microplastics physical data should be standardised by their size, type and colour. There are several pre-existing methods for reporting microplastic characteristics, therefore, to ensure comparability, particularly with long-standing datasets, it is recommended that photographs of the different types of microplastic, along with a scale bar, are included in reporting, e.g., as supplementary information in published material.

Physical properties

Reporting on the physical properties of microplastics is essential in the identification and confirmation of microplastics, while also providing important information on potential sources of contamination. However, care must be taken to avoid human error or bias when measuring the size, morphology, type, and colour of individual microplastics. Automated image analysis methods can be much faster and more reliable than human assessment, but require photographs/images taken under consistent lighting conditions at a known scale and of suitable resolution to ensure accurate and reproducible measurements of

physical parameters. Automated image analysis requires plastics to be photographed on a background that is sufficiently distinct in terms of colour and brightness so that each object can be reliably detected, or under different light sources (i.e., UV light). The use of these resources should be considered to mitigate observer bias, however, they may not be readily available to all.

Size

Plastic size is an essential physical attribute for reporting, as it will dictate how plastic interacts with the environment and wildlife. The size of objects can be described in many ways, but for plastics size usually refers to the length of the longest axis (the maximum Feret diameter) and is grouped into different size ranges (Table 2). The simplest method is to categorise the plastic object using visual assessment against a scale bar. For larger objects (> 1mm) length can be measured more precisely using callipers, but this can be time-consuming for large quantities of plastics. If photographs or microscopy images with a known scale are available, automated image analysis software can be used to instantly and precisely measure multiple different size parameters for each detectable object in the image. When reporting size parameters, always specify which parameter was used (e.g. maximum Feret diameter, or major diameter from elliptical approximation) and present size distributions as well as category counts, where possible. It is essential to report the minimum size category in which the approaches used can accurately detect, in line with the size categories in Table 11.

Table 11. Size classifications used to categorise plastic, from macroplastics (> 2.5 cm) to microplastics (1 µm* - 5 mm), as well as nanoplastics (< 1µm), which require specialised procedures for detection.

Plastic category	Size range
Macroplastics	>2.5 cm
Mesoplastics	0.5 - 2.5 cm
Visible microplastics	1 - 5 mm
Microscopic microplastics	1 µm* - 1 mm
Nanoplastics (outside of the scope of this manual)	< 1 µm

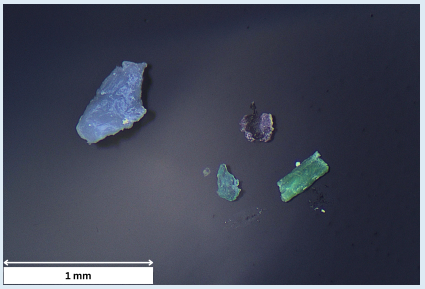
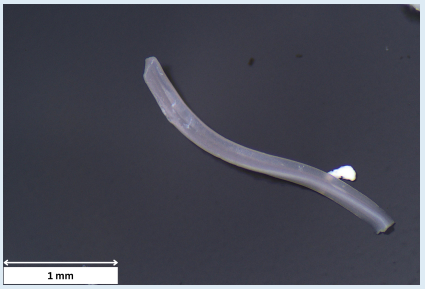
* Note: This field manual only focuses on microplastics > 20 µm.

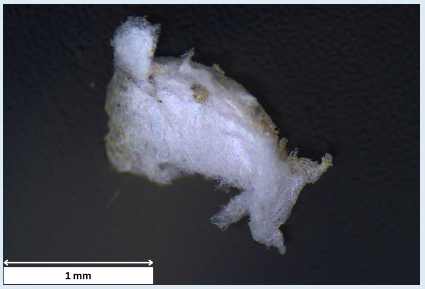
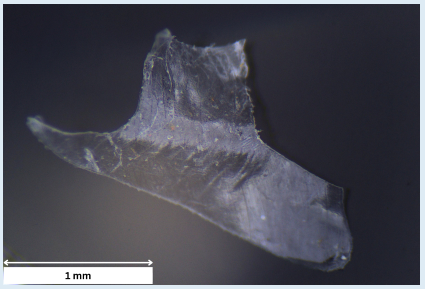
Type

Plastic type is potentially indicative of its original form and function (e.g., pellets used as raw material vs fibres from textiles), though many microplastics are highly fragmented, degraded and show little physical resemblance to the original object. Defining the plastic

type is dictated by the apparent shape, morphology and texture, assessed visually, as well as tactility (Table 12). Microplastics should be categorised to one of the most common overarching plastic types, and if more details of source are evident (e.g., artificial turf) this should be reported additionally. If photographs/images are available, automated image analysis software can provide some indication by measuring various shape parameters (e.g., regular, irregular, round) of each object (Valente et al., 2023), however, this approach is generally limited to differentiating pellets (small, round) from fragments (small, angular) from fibres (long, thin). The specific type parameters and the thresholds for differentiating each type must be reported.

Table 12. Classification of various plastic types observed in environmental samples. Types include fibres, fragments, films, beads, and others, providing insights into the diverse forms of plastic pollution. Photo credit: Thomas Crutchett.

Type	Description	Photo example
Pellets	Small, plastic particles, from raw materials in industrial plastic production (plastic feedstock). Normally bigger in size (e.g., nurdles) but also includes microbeads and powder. Scale is 1 mm.	
Fragments	Originate from the breakdown of larger hard plastic objects, like bottles or containers. These fragments can take various shapes and sizes, be hard or soft, often with irregular edges. An example of an irregular shaped fragment can be tyre wear fragments.	
Filaments	Strands of synthetic materials. These are often shed from clothing, textiles, and fabrics during washing and wear (fibres) or strands of fishing line which may be in monofilament or braided forms (line). Filaments can generally bend and are of uniform thickness across their length.	

Foams	Expanded plastic foam materials, like foam cups, packaging and insulation. These particles are lightweight and will compress if squeezed.	
Films	Originate from larger soft plastic materials, such as plastic bags and packaging. They are typically thin (and often transparent) and flexible, resembling miniature sheets or layers of plastic.	

Colour

The colour of microplastics may be indicative of the original object or the extent of weathering or contamination (e.g., absorption of contaminants and biofilm), and may also influence the likelihood of ingestion by wildlife. Colour has conventionally been reported by assigning each plastic to a colour category based on visual assessment, but human judgment of colour is extremely subjective and the list of colour categories used is far from consistent between different studies. Recent efforts to standardise the reporting of plastic colour recommend using the following six categories: white, yellow, red, green, blue, and black (Provencher et al., 2017), or by comparison to 120 standard Pantone colours (Martí et al., 2020). These standard categories are inherently imprecise and can still be inconsistently applied by different human observers. If photographs/images are available, the average colour of a plastic object should instead be measured in terms of red, green, and blue (RGB) values or hue, saturation value (HSV) as acquired by the camera. RGB values are more precise and less subjective than colour categories and provide semi-continuous data (e.g. suitable for studying trends such as discolouration), but care must be taken to ensure the images are taken under consistent white lighting to correctly reproduce true colours. For larger (>1 mm) plastics that can be imaged using conventional photography, a photographic colour reference card imaged alongside the plastics can provide a point of calibration ensuring greater consistency in colour reproduction. If a colour reference card cannot be included, it is essential to report the lighting used (source, colour, temperature, etc.). It is important to note that for transparent and semi-transparent

plastics, apparent colour may also be affected by the colour of the background.

Chemical characterisation

Due to challenges in identifying some microplastics using visual methods, especially small and/or transparent items, chemical analysis should be used to validate the identification of plastic, and confirm polymer composition and the anthropogenic nature of the particles. Importantly, polymer identification can also help identify the potential sources of microplastic, which in turn can influence regulation and policy. To confirm and validate that all retrieved particles were plastics, the preference should be that 100% of items are assessed. In instances where the number of putative microplastics is high and resources are not commensurate, then subsampling should match the resources available. If subsampling does occur, it is essential that the percentage of microplastics tested is reported, as well as the method used to select this subset and how representative this may be of the entire sample. This is an essential step to validating that the retrieved putative microplastics are indeed plastic, particularly for smaller microplastics.

There are several spectroscopy and spectrometry options for the chemical identification of plastics, with the method employed often dependent on the available equipment and the research question (Table 13). Although any chemical information about the microplastic is useful, Fourier-Transform Infrared Spectroscopy (FTIR) and Raman spectroscopic techniques are routinely used.

The chemical identification of microplastics involve comparisons of measured spectroscopic/spectrometric data compared to reference libraries of known materials. Reference libraries are usually a built-in functionality of commercial software used to operate the equipment, but the selection and breadth of available libraries vary between manufacturers and equipment. Furthermore, identification of plastics by spectroscopic FTIR and Raman methods can be complicated by chemical weathering, which alters the signature of the base material, and by biological and chemical contamination, which may introduce substantial secondary peaks in FTIR measurements and background fluorescence in Raman measurements (Fernández-González et al., 2021; Phan et al., 2022). Because library matching is a mathematical comparison, it may be misled by the appearance of altered or additional peaks, or by changes in background. To avoid erroneous assignments of weathered/degraded/contaminated plastics to inappropriate materials, matches should be assessed by an expert. Always consider whether the closest matching material spectrum is appropriate given the observed peaks, as well as the type, colour, and texture of the sample.

Table 13. Summary table of polymer identification instrumentation and their advantages, limitations, and minimum size that can be sampled.

Machine	Size limit	Advantages	Limitations
µ-FTIR	20 µm	- Sample retained - Broadly applicable to a range of samples - Commercial and open-source libraries of polymer spectra available	- Time intensive
ATR-FTIR	20 µm	- Sample retained - Broadly applicable to a range of samples - Commercial libraries of polymer spectra available	- Time intensive - Soft/brittle samples may be damaged - Can only be done efficiently for larger microplastics (> 500 um)
Raman	1 µm	- No contact and sample retained - Broadly applicable to a range of samples	- Background fluorescence from plastic additives and/or contaminants can increase identification complexity - Time intensive
Pyr-GC/MS	Independent of particle size	- Broadly applicable to a range of samples - Suitable for simultaneous identification and mass quantification of plastics - Reduces sample treatment	- Polymer types and abundance cannot be associated with particle size, type, shape, color, or numbers - Costly
LDIR	20 µm	- Non-destructive - Quicker analysis	- Costly

Quality Assurance (QA) and Quality Control (QC)

Visible microplastics (1 mm - 5 mm)

For larger microplastics, quality assurance and quality control are not as elaborate as when identifying microplastics < 1 mm. The hot needle test can be appropriate to use for larger visible microplastics if looking to only confirm if an item is plastic or not. However, it is important to still limit potential extraneous contamination during processing when possible and follow the same steps as if searching for small microplastics (see below).

Microscopic microplastics (1 µm - 1 mm)

During sample collection, it is important to reduce and where possible account for all sample contamination with extraneous microplastics (i.e., plastics coming from sampling gear or air). This may be harder in some environments than others (e.g., if sampling on a boat). The effectiveness of various procedures to limit contamination have recently been investigated (Jones et al. 2024). In brief, four different sources of plastic contamination in laboratory procedures were assessed, including water (e.g., Milli-Q), airflow (e.g., fume hood), dust and consumables (e.g., glassware). Findings indicate water, air flow and dust can be significant sources of contamination, and should therefore be given careful consideration during study design. While ultrapure water (Milli-Q) and reverse osmosis were found to be the least contaminated sources of water for experiments, some contamination was detected in all water types. Glass or stainless steel equivalents often used to replace plastic consumables, as well the aluminium foil used to reduce airborne contamination can also be significant sources of contamination. Given how ubiquitous contamination was, procedural blanks (more below) and the benefits/risks of alternate approaches, such as washing plastic consumables with detergent so they can potentially be reused are tested and discussed at length in Jones et al. (2024). However, as QA/QC procedures can vary depending on the aims of the study (e.g., microplastics vs nanoplastics) it is strongly recommended that researchers familiarise themselves with the specific reporting requirements and methods relating to sources of contamination, mitigation, and implementation of controls (see also recommendations in Jones et al. (2024))

At a minimum, all experimental equipment should be rinsed 2-3 times in filtered/ultrapure water (e.g., Milli-Q) before use. Avoid the use of aluminium foil to cover samples and instead use glass lids (i.e., watch glass, Petri dish lid). Sample processing should be performed in a biological safety cabinet (BSC) or similar laminar flow cabinet (LAF bench),

and not in a fume hood. BSC and LAF cabinets provide a sterile and particle-free workspace by directing filtered air over the work surface, ensuring protection for sensitive materials and experiments.

It is desirable that glassware is also decontaminated using an acid wash [e.g., diluted nitric acid solution (HNO_3) or diluted hydrochloric acid (HCl)], following standard operating procedures for decontamination of glassware in the laboratory.

Frequent cleaning of the lab area (not just benches) with 70% ethanol and a lint-free cloth should be undertaken to reduce the build-up and distribution of laboratory dust. Other precautions to reduce the likelihood of plastic particles being carried into the lab on operators include: the use of a sticky mat at the entrance of the lab, restricting foot traffic in the laboratory space and limiting the number of personnel working in the immediate vicinity of the workspace. There has been much discussion of researchers limiting the use of synthetic clothing while working in the lab or using brightly coloured synthetics when synthetics cannot be avoided (to help with the identification of fibres). This is a precautionary approach. At present, there are no data to confirm this is beneficial and is an area that could benefit from further investigation.

A series of blanks and controls should be used through all stages of the methods. A list is provided below, and the reader is also referred to Jones et al. (2024) for a discussion of experimental design:

Field blanks: These are taken from field items used to perform the sample collection and capture contamination from field sampling gear (i.e., ropes, nets, hoses), sampling and storage containers, and materials from the vessel (i.e., paint, decking) or operator (i.e., clothing and PPE). Field blanks help assess whether contamination has occurred during the field sampling process.

Laboratory blanks: Similar to field blanks, these are taken from laboratory items used to perform the sample processing and capture contamination from laboratory gear and materials (i.e., paint, stoppers, O-rings, labels) or operator (i.e., clothing and PPE). Lab blanks help assess whether contamination has occurred during the sample processing.

Procedural blanks: These are matrix free blanks that are treated in the same manner as environmental samples, undergoing all processing reagents and run through all laboratory sample processing steps. They are generally run at the beginning, during and at the end of a sample set. They help identify contamination introduced during laboratory processing.

Airborne contamination controls: These controls capture any contamination that may be in the air during the collection and processing of samples.

Positive controls: These involve the use of known microplastic standards of different sizes, types, and compositions. They are used to calibrate instruments, validate analytical methods, and ensure the accuracy of microplastic recovery, identification and quantification.

Reporting and Data Release

These methods will differ among environments so refer to the relevant section:

Water

All data should aim to be publicly released, unless circumstances restrict this (e.g., confidentiality or embargo, grant agreement, etc.). In situations where data cannot be shared, the metadata should still be made available. (Ideally, four key metrics should be reported for microplastic from environmental water samples.

1. **Load**
   This provides information on how much microplastic was detected per collection, and enables comparisons of microplastic load with other work to better understand how much plastic is in coastal and marine environments, and whether levels can change in space and time. Reporting units (e.g., MPs/m^3, MPs/area or MPs/transect) should be considered during the sampling design phase and checked for applicability to the research question. Although reporting units are often related to specific research goals, providing supplementary information with the raw data or transformed data in complementary units is recommended to allow broadscale comparisons among studies.
2. **Physical characteristics**
   This provides information about the types of microplastics present in the environment and is essential given the diversity of microplastic, types, sizes and colours. This information can provide both knowledge on risks associated with the presence of different microplastic types and assist managers in making informed decisions to reduce the input of key plastic items into waterways.
3. **Chemical characteristics**
   This provides information on polymer type, the presence of additives, and other chemicals, as well as potential evidence of weathering (e.g., age, biofilm, degradation).
4. **Contextual information**

Information, particularly describing local and regional environmental and biotic variables can help researchers make like-for-like comparisons. For example, for seawater samples, factors such as wind and swell direction, depth and presence of oceanographic features (e.g., slicks) at the collection site, as well as the location of potential point-sources of contamination (e.g., Wastewater Treatment Plant outflows, sewer or drainwater outflows) can influence plastic input rates, and can inform possible sources and fates.

Reporting microplastic load

It is essential that microplastic data are reported in number per area or volume, with defining morphological characteristics such as type, colour, size, texture, and polymer type also included. Where possible, data for larger microplastics should also be reported in mass; noting small microplastics (<1 mm) can be difficult to weigh. However, it is recommended they are weighed where there is high loading in the sample and they can be combined as an indicator of plastic mass, while reporting counts of polymer types. Additionally, reporting the measure of variability and replication is also essential.

Essential parameters:

- Average number (±SD or SE) of MP per area (# particles km^{-2}, # particles m^{-2})
- Average number (±SD or SE) of MP per volume (# particles L^{-1})
- Average mass (±SD or SE) of MP per area (g MP km^{-2}, g MP m^{-2})
- Average mass (±SD or SE) of MP per volume (g MP L^{-1}, g MP m^{-3})

Desirable parameters:

- Average number (±SD or SE) polymers per volume (e.g., # of nylon fibres m^{-3})
- Average number (±SD or SE) of MP size category per volume (e.g., # of MPs 20-300 µm m^{-3})
- Average number (±SD or SE) of MP colour category per volume (e.g., # of blue MPs m^{-3})

Reporting physical and chemical characteristics

Harmonisation in the reporting of physical data is challenging primarily due to the heterogeneity of microplastics, however, there is essential basic morphological data that is required for early identification and verification of polymer assignment (i.e., through the elimination of potential contaminants) and desirable information that is project specific.

Essential parameters:

- Maximum length
- Maximum width perpendicular to the length
- Surface area (of the item sitting flat)
- Mass (for visible microplastics > 1mm)
- Colour (RGB and HSV for larger microplastics)
- Type (e.g., pellet, fragment, film, filament, foam)
- Polymer (for microscopic 1 µm - 1 mm microplastics)
- Detection limits for the count and minimum microplastic size

Desirable parameters:

- Mass per item and/or type
- Polymer (for visible microplastics >1 mm)
- Percentage of microplastics and natural particles present

Reporting contextual information

It is important to consider and report information on the environmental variables at the time of collection. Where possible this information should be collected at the time of sampling, but some data and information can be calculated in hindsight (distance travelled using GPS location data) or attained through in situ sensors, but all should be recorded where feasible.

General site information

- Location (including latitude and longitude coordinates)
- Location description [physical features, biotic features of the area (e.g., algal coverage) and habitat type]
- Date of data collection; season
- Recent weather events (e.g., dust storms, cyclones, floods) and conditions during sampling event (e.g., temperature, wind, rain)
- Wind speed and direction
- Swell height and direction
- Collection depth, temperature and salinity
- Tide
- Vessel speed
- Tow direction and/or depth profile
- Proximity to urban and/or industrial areas
- Proximity to river streams and/or estuaries
- Proximity to wastewater treatment plants and number of outflows, drain or stormwater outlets and other potential point sources of contamination

- Presence of floating macro and meso plastics in the immediate area of collection or in the sample
- Presence of organisms in the immediate area of collection or in the sample
- Presence of oceanographic features as indicators of marine processes (e.g., sea slicks) in the immediate area of collection

Sediment

All data should aim to be publicly released, unless circumstances restrict this (e.g., confidentiality or embargo, grant agreement, etc.). In situations where data cannot be shared, the metadata should still be made available. Ideally, four key metrics should be reported for microplastic from sediment samples.

1. **Load**
   This provides information such as how much microplastic was detected in the sediment and enables comparisons of microplastic load with other work to better understand how much plastic is in coastal and marine environments. Reporting units (e.g., number or mass of microplastics per cubic metre, sampled area or transect) should be considered during the design phase and checked for applicability to the research question. Although reporting units are often related to specific research goals, providing supplementary information with the raw data or transformed data in complementary units is recommended to allow broadscale comparisons among studies.
2. **Physical characteristics**
   This provides information about types of microplastics present in the environment, and is essential given the diversity of microplastic, types, sizes, and colours. This information can provide both knowledge on risks associated with the presence of different microplastic types and assist managers in making informed decisions to reduce the input of key items.
3. **Chemical characteristics**
   This provides information on polymer type, presence of additives and other chemicals, as well as potential evidence of weathering (e.g., age, biofilm, degradation).
4. **Contextual information**
   Information, particularly describing environmental and biotic variables can help researchers make like-for-like comparisons. For example, for coastal sediment samples, factors such as wind and swell direction, local hydrodynamics, sedimentology, and location of potential point-sources of contamination (e.g., WWTP outflows, sewer or drainwater outflows) can influence plastic input rates and can inform possible sources and fates.

Reporting microplastic load

It is essential that microplastic load is reported in units commensurate with the method, e.g., number per mass in grab samples, number per area in quadrat and transect samples. Reporting mass of microplastic is desirable. Where possible, is it recommended that data for larger microplastics should also be reported in mass; noting small microplastics (<1 mm) can be difficult to weigh, particularly if they are damaged during spectroscopy (GESAMP, 2019). However, it is recommended they are weighed where there is high loading in the sample and they can be combined as an indicator of plastic mass, while reporting counts of polymer types.Additionally, reporting the measure of variability and replication is also essential.

Essential parameters:

- Average number (±SD or SE) of MP per area (# particles km^{-2}, # particles m^{-2})
- Average number (±SD or SE) of MP per volume (# particles m^{-3})
- Average number (±SD or SE) of MP per mass (# particles kg^{-1} dry sediment)
- Average mass (±SD or SE) of MP per area (g MP km^{-2}, g MP m^{-2})
- Average mass (±SD or SE) of MP per volume (g MP L^{-1}, g MP m^{-3})
- Average mass (±SD or SE) of MP per mass (g MP g^{-1})

Desirable parameters:

- Average number (±SD or SE) polymers per volume (e.g., # of nylon fibres m^{-3})
- Average number (±SD or SE) of MP size category per volume (e.g., # of MPs 20-300 µm m^{-3})
- Average number (±SD or SE) of MP colour category per volume (e.g., # of blue MPs m^{-3})

Reporting physical and chemical characteristics

Harmonisation in the reporting of physical data is challenging primarily due to the high heterogeneity between microplastics, however, there is essential morphological data that is needed for early identification and verification of polymer assignment (i.e., through elimination of potential contaminants) and desirable information that is project specific.

Essential parameters:

- Maximum length
- Maximum width perpendicular to the length
- Surface area (of the item sitting flat)

- Mass (for visible microplastics > 1mm)
- Colour (RGB and HSV for larger microplastics)
- Type (e.g., pellet, fragment, film, filament, foam)
- Polymer (for microscopic 1 µm - 1 mm microplastics)
- Detection limits for the count and minimum microplastic size

Desirable parameters:

- Mass per item and/or type
- Polymer (for visible microplastics >1 mm)
- Percentage of microplastics and natural particles

Reporting contextual information

Supratidal, intertidal, and subtidal zones can have a variety of ecosystems and habitats. It is important to consider and report information that fully describes the sampling habitat, including environmental variables, to maximise comparisons among sites and studies, namely on the following variables:

General site information

- Location (including latitude and longitude coordinates)
- Location description [physical features, biotic features of the area (e.g., algal coverage, crab holes, oyster beds) and habitat type]
- Date of data collection; season
- Recent weather events (e.g., dust/sandstorms, cyclones, floods) and conditions during sampling event (e.g., temperature, wind, rain, tide)
- Proximity to urban and/or industrial areas
- Proximity to river streams and/or estuaries
- Proximity to wastewater treatment plants and number of outflows, drain or stormwater outlets and other potential point sources of contamination
- Number of waste bins
- Proximity to beach infrastructures (e.g., surf clubs, cafes, restaurants, shopping precincts, nightclubs)

Intertidal sediment

- Type of sediment, determined by granulometry

- Beach slope (including sand migration)
- Wind speed and direction
- Amount of macro and meso marine litter in the vicinity area of collection
- Presence of organisms in the area of collection or in the sample
- Tide (last high tide)

Subtidal sediment

- Type of sediment, determined by Granulometry and %TOC
- Wave height
- Collection depth, temperature, and salinity
- Amount of floating macro and meso plastics in the vicinity of area of collection
- Presence of organisms in the area of collection or in the sample
- Hydrodynamic profile

Note, not all of this information needs to be collected at the time of sampling but can be recorded in hindsight (using GPS location data), or attained through in situ sensors, but all should be recorded.

Biota

All data should aim to be publicly released, unless circumstances restrict this (e.g., confidentiality or embargo, grant agreement, etc.). In situations where data cannot be shared, the metadata should still be made available. Ideally, four key metrics should be reported for microplastic in biota samples.

1. **Load**
   This provides information such as how much microplastic was detected in biota, and enables comparisons of microplastic load with other work to better understand how much plastic is in biota across different environments, and whether levels are changing. Reporting units (e.g., average number of microplastics per organism, or tissue weight) should be considered during the sampling design phase and checked for applicability to the research question. Although reporting units are often related to specific research goals, providing supplementary information with raw data or transformed data in complementary units is recommended to allow broadscale comparisons among studies.
2. **Physical characteristics**
   This provides information on the types of microplastics present in the environment, and is essential given the diversity of microplastic types, sizes and colours. This information can both provide knowledge on risks associated with the uptake and presence of different microplastic types and assist managers in making informed

decisions to reduce the input of key items.

3. **Chemical characteristics**
   This provides information on polymer type, presence of additives and other chemicals, as well as potential evidence of weathering (e.g., age, biofilm, degradation).
4. **Contextual information**
   Information, particularly around environmental and biotic variables, can help researchers make like-for-like comparisons. For example, for biotic samples, colony size, scat photos, other fishery species collected alongside the target species, and samples of plastic items from the vessel, fishing gear, storage containers can all be used to interrogate and contextualise the microplastics data.

Reporting microplastic load

Microplastics must be reported in the number of microplastics per organism and/or per weight of tissue, with defining morphological characteristics such as type, colour, size, texture, and polymer type also included. Where possible, data for larger microplastics should also be reported in mass; noting small microplastics (< 1 mm) can be difficult to weigh. However, it is recommended they are weighed where there is high loading in the sample and they can be combined as an indicator of overall plastic mass, while also reporting counts of polymer types. Additionally, reporting the measure of variability in microplastic load and sample replication is also essential.

Essential parameters:

- Average number (±SD or SE) of microplastics extracted from all organisms (# particles per organism)
- Average number (±SD or SE) of microplastics extracted from organisms found to contain plastic (# particles per contaminated organisms)
- Total number of individuals analysed
- Number of individuals containing at least one microplastic piece (reported as a percentage of frequency of occurrence)

Desirable parameters:

- Mass per microplastic item and/or type
- Polymer (for all microplastics)

It is also important to report, when possible, the different physical characteristics of the microplastic pieces when reporting results (e.g., 10 nylon fibres L^{-1}; 4 grams of nurdles m^{-3}). This extra information is important in allowing appropriate comparisons between data sets,

as well as providing useful data for higher-level users (e.g., policy makers).

Reporting microplastic physical characteristics

Harmonising the reporting of physical data is challenging due to the heterogeneity of microplastics (Refer to Introduction and scope); however, there is essential morphological data that are needed to contextualise microplastics that have been taken up by biota. For example, Antarctic krill can significantly alter the physical size of ingested spherical microplastics (from 31.5 µm to 1 µm in diameter; (Dawson et al., 2018)). If smaller items are found, then one would expect larger items of the same polymer type to be in the surrounding water. While there is little evidence that the digestion system or associated microbiome can decompose microplastics (plastics are inert due to their low chemical reactivity), recording details of the specific compartment (i.e., stomach that contains digestive enzymes vs cloaca that is high in organic waste) in which the microplastic was retrieved is important.

Essential parameters:

- Maximum length
- Maximum width perpendicular to the length
- Surface area (of the item sitting flat)
- Mass (for visible microplastics > 1mm)
- Colour (RGB and HSV for larger microplastics)
- Type (e.g., pellet, fragment, film, filament, foam)
- Polymer (for microscopic 1 µm - 1 mm microplastics)
- Biotic compartment
- Detection limits for the count and minimum microplastic size

Desirable parameters:

- Mass per item and/or type
- Polymer (for all microplastics)
- Percentage of microplastics and natural particles present.

Metadata and contextual information

It is important to consider and report information on the following environmental variables at time of collection. Where possible, this information should be collected at the time of sampling, but some data can be calculated after sampling (e.g., GPS location data). Information pertaining to the biology and anatomy of the target species should also be

gathered to understand the impacts of the microplastics exposure.

General site information

- Location (including latitude and longitude coordinates)
- Location description (physical features, features of the area, e.g., algal coverage)
- Habitat type including presence of prey items and predators
- Date of data collection; season
- Recent weather and weather conditions during sampling event (e.g., temperature, wind, rain, recent weather events such as dust storms, cyclones, floods)
- Wind speed and direction
- Wave height
- Tide
- Collection depth, temperature and salinity
- Proximity to urban and/or industrial areas
- Proximity to river streams and/or estuaries
- Proximity to wastewater treatment plants and number of outflows, drain or stormwater outlets and other potential point sources of contamination
- Presence of macro, meso and microplastics in the immediate environment (i.e., water column, sediment)
- Target species behaviour

Air

All data should aim to be publicly released, unless circumstances restrict this (e.g., confidentiality or embargo, grant agreement, etc.). In situations where data cannot be shared, the metadata should still be made available. Ideally, four key metrics should be reported for microplastic from air samples.

1. **Load**
   This provides information such as how much microplastic was detected per collection and enables comparisons of microplastic load with other work to better understand how much plastic is in the environment. Reporting units (e.g., MPs m^{-3}, particle m^{-2} d^{-1}) should be considered during the sampling design phase and checked for applicability to the research question. Although reporting units are often related to specific research goals, providing supplementary information with the raw data or transformed data in complementary units is recommended to allow broadscale comparisons among studies.
2. **Physical characteristics**
   This provides information on the types of microplastics present in the environment, and is essential given the diversity of microplastic types, sizes and colours. This

information can provide both knowledge on the risks associated with the presence of different microplastic types and assist managers in making informed decisions to reduce the input of key plastic items into the environment.

3. **Chemical characteristics**
   This provides information on the polymer types, presence of additives or other chemicals, as well as potential evidence of weathering (e.g., age, biofilm, degradation).
4. **Contextual information**
   Information, particularly describing local and regional environmental and biotic variables can help researchers make like-for-like comparisons. For example, for air samples, wind strength and direction, or location of potential point-sources of contamination (e.g., industry) can influence plastic input rates.

Reporting microplastic load

It is essential that microplastic load in air is reported as number per volume or area, with defining morphological characteristics also included, such as type, colour, size, texture, polymer type. Where possible, data for larger microplastics should also be reported in mass; noting small microplastics (<1 mm) can be difficult to weigh. However, it is recommended they are weighed where there is high loading in the sample and they can be combined as an indicator of plastic mass, while reporting counts of polymer types. Additionally, reporting the measure of variability and replication is also essential.

Essential parameters:

- Average number (±SD or SE) of MPs per volume (# particles m^{-3} for active sampling)
- Average number (±SD or SE) of MPs per area (# of particle.m^{-2} d^{-1} for passive sampling)
- Average mass (±SD or SE) of MP per volume (g MP m^{-3})
- Average mass (±SD or SE) of MP per area (g MP m^{-2} d^{-1})

Desirable parameters:

- Average number (±SD or SE) polymers per volume (e.g., # of nylon fibres m^{-3} for active sampling)
- Average number (±SD or SE) polymers per area (e.g., # of nylon fibres m^{-2} d^{-1} for passive sampling)
- Average number (±SD or SE) of MP size category per volume (e.g., # of MPs 20-300 m^{-2} d^{-1} for passive sampling)
- Average number (±SD or SE) of MP size category per area (e.g., # of MPs 20-300 µm m^{-3})

- Average number (±SD or SE) of MP colour category per volume (e.g., # of blue MPs m^{-3})
- Average number (±SD or SE) of MP colour category per area (e.g., # of blue MPs m^{-2} d^{-1} for passive sampling)

For conversion between units, we recommend using the formula in Leusch and Ziajahromi (2021).

Reporting physical and chemical characteristics

The harmonisation of physical data is challenging primarily due to the high heterogeneity between microplastics; however, there is essential morphological data that is required for early identification and verification of polymer assignment (i.e., through elimination of potential contaminants) and desirable information that is project-specific.

Essential parameters:

- Maximum length
- Maximum width perpendicular to the length
- Surface area (of the item sitting flat)
- Mass (for visible microplastics > 1mm)
- Colour (RGB and HSV for larger microplastics)
- Type (e.g., pellet, fragment, film, filament, foam)
- Polymer (for microscopic 1 µm - 1 mm microplastics)
- Detection limits for the count and minimum microplastic size

Desirable parameters:

- Mass per item and/or type
- Polymer (for visible microplastics >1 mm)
- Percentage of microplastics and natural particles

Reporting contextual information

It is important to consider and report information on the environmental variables at the time of collection. Where possible, this information should be collected at the time of sampling, but some data can be calculated in hindsight (e.g., volume filtered).

General site information

- Location (including latitude and longitude coordinates)

- Location description (including physical features of the area)
- Date of data collection; season
- Weather conditions during sampling event (e.g., temperature, wind, rain)
- Recent weather events such as dust storms, cyclones, floods or others that may influence microplastic dispersal.
- Wind speed and direction
- Proximity to urban and/or industrial areas
- Proximity to river streams and/or estuaries
- Proximity to industrial infrastructure
- Proximity to roads

Note, not all of this information needs to be collected at the time of sampling, but can be recorded in hindsight (using GPS location data), but all should be recorded.

Checklist of methods and result reporting

Table 14. Checklist of necessary information for conducting and reporting environmental microplastics research, adapted from Cowger et al. (2020). * indicates compulsory items to be reported.

Reported (✓)	Action Item	Define the action or information determined by you and your research team *(some examples provided)*
General considerations		
	*** Has the research question been defined**	***i.e., Type of microplastics in sea surface waters in the Great Australian Bight***
	*** Which field manual will you follow?**	***i.e., Water***
	Are there any existing standard protocols suitable to investigate your research question?	***i.e., AIMS Australia, CSIRO Australia***
Selection of a sampling design		
	Sampling effort required (e.g., power analysis)	***i.e., Power analysis completed indicated we needed a minimum of 3 replicates***

	*** Location**	***i.e., Nuyts Archipelago, Great Australian Bight***
	*** Number of samples to collect per location**	***i.e., Five***
	*** Number of replicates**	***i.e., Three (therefore, n = 3, N = 15)***
	Is my sampling design representative of the habitat/compartment that I wish to answer a research question about?	***i.e., Yes***
	Randomised sampling?	***i.e., Yes***
Materials & Equipment used		
	Devices/equipment & manufacturers	***i.e., Rosette sampler niskin bottles***
	Software	***i.e., N/A***
	How were the devices and software calibrated	***i.e., N/A***
Quality Assurance & Quality Control		
	Defined controls and replication (e.g., field blanks, procedural blanks, airborne contamination control, positive controls, recovery tests)	***i.e., Field blanks – 5 per site, Procedural blanks – every 10 samples***
	Defined and clear limit of detection (e.g., microplastic size constraints)	***i.e., Detection limit 20 mm***
	*** Actions taken for contamination mitigation**	***i.e., For field procedures cotton clothing was worn by operators and niskin bottles were rinsed with ultrapure water between sample collection. For laboratory procedures a laminar hood was used, and 100% cotton lab coats,***

		with leather boots worn in the laboratory. Reported in report
	Control correction procedure/s on the environmental data	*i.e., N/A*
	Laboratory and equipment cleaning procedures	*i.e., Clean room. All labware were washed with ultrapure water and dried in laminar flow*
	Reagent purification methods	*i.e., All reagents were filtered with 0.45 mm filter paper*
Field Sampling		
	*** Record of field location (including GPS coordinates), sampling date, and time**	*i.e., Yes GPS for sites recorded in excel spreadsheet with dates and time*
	Records of additional environmental conditions where applicable (e.g., air temperature, wind, tides, water temperature, pH, and salinity)	*i.e., Yes see GPS spreadsheet for recorded water and air temperature and wind*
	Surface area or volume of sample collected from environmental matrices, with appropriate units	*i.e., 10 L per bottle*
	*** Composition of the sample**	*i.e., Water*
	Description of sampling equipment and relevant dimensions used to collect the samples, and any cleaning procedures between sample collection	*i.e., CTD rosette sampler brand x. Niskin bottles were rinsed with ultrapure water between sample collection*
	*** Depth and/or position the sample was collected from**	*i.e., 10 m below surface, >1 km from shore*

	*** Description of other sampling methodologies (e.g., vessel speed when towing a net, direction of travel, speed and direction of current, amount of water filtered)**	***i.e., vessel speed 0 knots, wind 10 knots SW,***
	Storage of samples from the field to the laboratory	***i.e., samples stored in cold, dark store room***
Laboratory Processing		
	Specification of reagents	***i.e., per analysis grade***
	Specification on the laboratory equipment used and all relevant details	***i.e., Leica M80 stereo microscope, Whatman glass fibre filters***
Process for separation of microplastics from environmental matrices		
	*** Sieving**	***i.e., Not required***
	Visual Separation	***i.e., Microscope***
	*** Density Separation (e.g., solution composition, concentration, volume per sample)**	***i.e., Not required***
	*** Chemical Digestion (e.g., chemicals used, duration of digestion, temperature, hear source, voume per sample, caveats of digestion method)**	***i.e., Not required***
	Filtration information	***i.e., Filtered materials through 0.45 mm glass fibre filter***
Microplastic Categorisation & Identification for Large & Small Microplastics		
Quantification		
	Determine method for quantification, refer to Table 10	***i.e., Manual counting***

	under "*Microplastic Quantification*"	
	*** Record of relevant information for quantification type**	***i.e., Visual identification: limit of detection was 20 mm***
Physical Characterisation		
	Type (refer to "*Physical Characterisation, Type table"* within this manual)	***i.e., Yes, see results spreadsheet***
	Colour	***i.e., Yes, see results spreadsheet***
	Size (refer to "*Physical Characterisation, Size table"* within this manual), where possible a more accurate information on individual particle sizes/volumes	***i.e., Yes, see results spreadsheet***
Chemical Characterisation		
	Determination of polymer type (refer to "*Chemical Characterisation"* within this manual)	***i.e., Yes, FTIR model x used, see results spreadsheet***
	*** Record of relevant information for determining polymer type (e.g., size limits, pre-processing steps, acquisition parameters, spectral matching software/techniques/or methodology)**	***i.e., micro-FTIR manual sample loading, Bruker library x, spectral range set between 3,900 and 650 cm^{-1} with 2,500 and 1,900 cm^{-1} excluded***
	Percentage of total microplastic samples tested	***i.e., 50%***
Data Management		
	Units and size dimensions reported, must be comparable	***i.e., Yes units per sample, weight and raw data were included in the report***

	Where possible, inclusion of average values with reports for variability (only possible with replication)	***i.e., Yes mean and standard error reported***
	Data submitted to an open access portal (e.g., AODN, Marine Plastics Portal)	***i.e., Yes data submitted to AODN***
	Raw data of counts to be included in the Supplementary Information or uploaded to suitable site (e.g. figshare). Aim to meet FAIR Guiding Principles for scientific data.	***i.e., Yes raw data included***
Reporting Results		
	*** The load (e.g. microplastic concentrations estimated, post inclusion of control data).**	***i.e., Yes microplastic load per litre reported.***
	*** The lower limit of size category**	***i.e., Only plastics >20 µm were recorded***
	The physical characteristics (e.g. type, colour, sizes)	***i.e., Yes – type, size and colour is reported***
	The chemical characteristics (presence and proportions of the different polymer types)	***i.e., Yes – polymer type for 50% of samples tested reported***
	Contextual information and metadata (such as environmental data)	***i.e., Yes included in the Materials and Methods section and supplementary information***
Other considerations		
	Can the results be published in an Open Access Journal?	***i.e., Yes***

Field Manual Maintenance

In accordance with the universal field manual maintenance protocol described in Chapter 1 of the Field Manual package, this manual was created in 2023 as part of Version 2.1.

The version control for Chapter 13 (field manual for microplastics) is below:

Version Number	Description	Date
1	There was no microplastics manual included in Version 1 of the field manual package	28 Feb 2018
2	There was no microplastics manual included in Version 2 of the field manual package	July 2020
3	Publicly released as Chapter 13 through online portal	May 2024

Acknowledgements

References

Adomat, Y., Kahl, M., Musche, F., Grischek, T., 2022. Evaluation of microplastics sediment sampling techniques—efficiency of common methods and new approaches. Microplastics and Nanoplastics 2, 27.

Australian Government, 2006. Standardised protocols for the collection of biological samples from stranded cetaceans, in: Department of Climate Change, E., Environment and Water (Ed.).

Azari, A., Vanoirbeek, J.A.J., Van Belleghem, F., Vleeschouwers, B., Hoet, P.H.M., Ghosh, M., 2023. Sampling strategies and analytical techniques for assessment of airborne micro and nano plastics. Environment international 174, 107885.

Brandt, J., Fischer, F., Kanaki, E., Enders, K., Labrenz, M., Fischer, D., 2021. Assessment of subsampling strategies in microspectroscopy of environmental microplastic samples. Frontiers in Environmental Science 8.

Caron, A., Thomas, C., Ariel, E., Berry, K., Boyle, S., Motti, C., Brodie, J., 2016. Extraction and identification of microplastics from sea turtles: method development and preliminary results.

Caron, A.G.M., Thomas, C.R., Berry, K.L.E., Motti, C.A., Ariel, E., Brodie, J.E., 2018. Ingestion of microplastic debris by green sea turtles (*Chelonia mydas*) in the Great Barrier Reef: Validation of a sequential extraction protocol. Marine Pollution Bulletin 127, 743-751.

Catarino, A.I., Thompson, R., Sanderson, W., Henry, T.B., 2017. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. Environmental Toxicology and Chemistry 36, 947-951.

Champley, S., Ekstrom, C., Dalgaard, P., Gill, J., Weibelzahl, S., Anandkumar, A., Ford, C., Volcic, R., De Rosario, H., 2020. pwr: Basic functions for power analysis.

Chen, G., Fu, Z., Yang, H., Wang, J., 2020. An overview of analytical methods for detecting microplastics in the atmosphere. TrAC Trends in Analytical Chemistry 130, 115981.

Cho, Y., Shim, W.J., Jang, M., Han, G.M., Hong, S.H., 2021. Nationwide monitoring of microplastics in bivalves from the coastal environment of Korea. Environmental Pollution 270, 116175.

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Marine Pollution Bulletin 70, 227-233.

Commonwealth Scientific and Industrial Research Organisation, Coordinating Body on the Seas of East Asia, U.N.E.P., 2022. Marine Litter Monitoring Methods Handbook, Part I.

Corazzola, G., Baini, M., Grattarola, C., Panti, C., Marcer, F., Garibaldi, F., Berio, E., Mancusi, C., Galli, M., Mazzariol, S., Fossi, M.C., Centelleghe, C., Casalone, C., 2021. Analysis of the gastro-intestinal tract of marine mammals: A multidisciplinary approach with a new multi-sieves tool. Animals 11, 1824.

Cowger, W., Booth, A.M., Hamilton, B.M., Thaysen, C., Primpke, S., Munno, K., Lusher, A.L., Dehaut, A., Vaz, V.P., Liboiron, M., Devriese, L.I., Hermabessiere, L., Rochman, C., Athey, S.N., Lynch, J.M., De Frond, H., Gray, A., Jones, O.A.H., Brander, S., Steele, C., Moore, S., Sanchez, A., Nel, H., 2020. Reporting guidelines to increase the reproducibility and comparability of research on microplastics. Applied Spectroscopy 74, 1066-1077.

Crutchett, T.W., Bornt, K.R., 2024. A simple overflow density separation method that recovers> 95% of dense microplastics from sediment. MethodsX, 102638.

Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M., Bengtson Nash, S.M., 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nat Commun 9, 1001.

Dawson, A.L., Motti, C.A., Kroon, F.J., 2020. Solving a sticky situation: Microplastic analysis of lipid-rich tissue. Frontiers in Environmental Science 8.

Dehaut, A., Cassone, A.-L., Frère, L., Hermabessiere, L., Himber, C., Rinnert, E., Rivière, G., Lambert, C., Soudant, P., Huvet, A., Duflos, G., Paul-Pont, I., 2016. Microplastics in seafood: Benchmark protocol for their extraction and characterization. Environmental Pollution 215, 223-233.

Di Fiore, C., Ishikawa, Y., Wright, S.L., 2024. A review on methods for extracting and quantifying microplastic in biological tissues. Journal of Hazardous Materials 464, 132991.

Ding, J., Sun, C., He, C., Li, J., Ju, P., Li, F., 2021. Microplastics in four bivalve species and basis for using bivalves as bioindicators of microplastic pollution. Science of the Total Environment 782, 146830.

Ding, J., Sun, C., Li, J., Shi, H., Xu, X., Ju, P., Jiang, F., Li, F., 2022. Microplastics in global bivalve mollusks: A call for protocol standardization. Journal of Hazardous Materials 438, 129490.

Dong, H., Wang, L., Wang, X., Xu, L., Chen, M., Gong, P., Wang, C., 2021. Microplastics in a Remote Lake Basin of the Tibetan Plateau: Impacts of Atmospheric Transport and Glacial Melting. Environmental Science & Technology 55, 12951-12960.

Egger, M., Schilt, B., Wolter, H., Mani, T., de Vries, R., Zettler, E., Niemann, H., 2022. Pelagic distribution of plastic debris (> 500 µm) and marine organisms in the upper layer of the North Atlantic Ocean. Scientific Reports 12, 13465.

Eriksen, M., Liboiron, M., Kiessling, T., Charron, L., Alling, A., Lebreton, L., Richards, H., Roth, B., Ory, N.C., Hidalgo-Ruz, V., 2018. Microplastic sampling with the AVANI trawl compared to two neuston trawls in the Bay of Bengal and South Pacific. Environmental Pollution 232, 430-439.

Eros, C., Marsh, H., Bonde, R., O'Shea, T., Beck, C., Recchia, C., Dobbs, K., Turner, M., Lemm, S., Pears, R., Bowater, R., 2007. Procedures for the salvage and necropsy of the Dugong (*Dugong dugon*), in: Authority, A.G.G.B.R.M.P. (Ed.), 2 ed. Australian Government Great Barrier Reef Marine Park Authority, Townsville.

Faul, F., Erdfelder, E., Lang, A.-G., Buchner, A., 2007. G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behavior Research Methods 39, 175-191.

Fernández-González, V., Andrade-Garda, J.M., López-Mahía, P., Muniategui-Lorenzo, S., 2021. Impact of weathering on the chemical identification of microplastics from usual packaging polymers in the marine environment. Analytica Chimica Acta 1142, 179-188.

Fisner, M., Majer, A.P., Balthazar-Silva, D., Gorman, D., Turra, A., 2017. Quantifying microplastic pollution on sandy beaches: the conundrum of large sample variability and spatial heterogeneity. Environmental Science and Pollution Research 24, 13732-13740.

Frias, J.P.G.L., Nash, R., 2019. Microplastics: Finding a consensus on the definition. Marine Pollution Bulletin 138, 145-147.

Galgani, F., Hanke, G., Werner, S., Oosterbaan, L., Nilsson, P., Fleet, D., Kinsey, S., Thompson, R.C., Van Franeker, J., Vlachogianni, T., 2013. Guidance on monitoring of marine litter in European seas. Publications Office of the European Union.

GESAMP, 2019. Guidelines for the Monitoring and Assessment of Plastic Litter in the Ocean, in: GESAMP Reports and Studies No.99 (Ed.), p. 123 pp.

Hamm, T., Barkhau, J., Gabriel, A.-L., Gottschalck, L.L., Greulich, M., Houiller, D., Kawata, U., Tump, L.N., Leon, A.S., Vasconcelos, P., Yap, V., Almeida, C., Chase, Z., Hurd, C.L., Lavers, J.L., Nakaoka, M., Rilov, G., Thiel, M., Wright, J.T., Lenz, M., 2022. Plastic and natural inorganic microparticles do not differ in their effects on adult mussels (Mytilidae) from different geographic regions. Science of the Total Environment 811, 151740.

Hengstmann, E., Tamminga, M., vom Bruch, C., Fischer, E.K., 2018. Microplastic in beach sediments of the Isle of Rügen (Baltic Sea) - Implementing a novel glass elutriation column. Marine Pollution Bulletin 126, 263-274.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environmental Science & Technology 46, 3060-3075.

Huang, Y., He, T., Yan, M., Yang, L., Gong, H., Wang, W., Qing, X., Wang, J., 2021. Atmospheric transport and deposition of microplastics in a subtropical urban environment. Journal of Hazardous Materials 416, 126168.

Ijsseldijk, L.L., Brownlow, A.C., Mazzariol, S., 2019. European best practice on cetacean post-mortem investigation and tissue sampling.

Jones, N.R., de Jersey, A.M., Lavers, J.L., Rodemann, T., Rivers-Auty, J., 2024. Identifying laboratory sources of microplastic and nanoplastic contamination from the air, water, and consumables. J Hazard Mater 465, 133276.

Karami, A., Golieskardi, A., Ho, Y.B., Larat, V., Salamatinia, B., 2017. Microplastics in eviscerated flesh and excised organs of dried fish. Scientific Reports 7, 5473.

Karlsson, T.M., Kärrman, A., Rotander, A., Hassellöv, M., 2020. Comparison between manta trawl and in situ pump filtration methods, and guidance for visual identification of microplastics in surface waters. Environmental Science and Pollution Research 27, 5559-5571.

Karlsson, T.M., Vethaak, A.D., Almroth, B.C., Ariese, F., van Velzen, M., Hassellöv, M., Leslie, H.A., 2017. Screening for microplastics in sediment, water, marine invertebrates and fish: Method development and microplastic accumulation. Marine Pollution Bulletin 122, 403-408.

Kedzierski, M., Le Tilly, V., Bourseau, P., Bellegou, H., César, G., Sire, O., Bruzaud, S., 2016. Microplastics elutriation from sandy sediments: A granulometric approach. Marine Pollution Bulletin 107, 315-323.

Keys, B.C., Grant, M.L., Rodemann, T., Mylius, K.A., Pinfold, T.L., Rivers-Auty, J., Lavers, J.L., 2023. New methods for the quantification of ingested nano- and ultrafine plastics in seabirds. Environmental Science & Technology 57, 310-320.

Knobloch, E., Ruffell, H., Aves, A., Pantos, O., Gaw, S., Revell, L.E., 2021. Comparison of Deposition Sampling Methods to Collect Airborne Microplastics in Christchurch, New Zealand. Water, Air, & Soil Pollution 232, 133.

Kroon, F., Motti, C., Talbot, S., Sobral, P., Puotinen, M., 2018. A workflow for improving estimates of microplastic contamination in marine waters: A case study from North-Western Australia. Environmental Pollution 238, 26-38.

Lanyon, J., Sneath, HL, Long, T, Bonde, RK, 2010. Physiological response of wild dugongs (*Dugong dugon*) to out-of-water sampling for health assessment. Aquatic Mammals 36(1).

Lavers, J.L., Hutton, I., Bond, A.L., 2021. Temporal trends and interannual variation in plastic ingestion by Flesh-footed Shearwaters (Ardenna carneipes) using different sampling strategies. Environmental Pollution 290, 118086.

Leusch, F.D.L., Ziajahromi, S., 2021. Converting mg/L to Particles/L: Reconciling the Occurrence and Toxicity Literature on Microplastics. Environmental Science & Technology 55, 11470-11472.

Li, J., Lusher, A.L., Rotchell, J.M., Deudero, S., Turra, A., Bråte, I.L.N., Sun, C., Hossain, M.S., Li, Q., Kolandhasamy, P., 2019. Using mussel as a global bioindicator of coastal microplastic pollution. Environmental Pollution 244, 522-533.

Li, J.Y.Q., Nankervis, L., Dawson, A.L., 2022. Digesting the indigestible: Microplastic extraction from prawn digestive tracts. Frontiers in Environmental Chemistry 3.

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2017. Sampling, isolating and identifying microplastics ingested by fish and invertebrates. Analytical Methods 9, 1346-1360.

Markic, A., Gaertner, J.C., Gaertner-Mazouni, N., Koelmans, A.A., 2019. Plastic ingestion by marine fish in the wild. Critical Reviews in Environmental Science and Technology.

Martí, E., Martin, C., Galli, M., Echevarría, F., Duarte, C.M., Cózar, A., 2020. The Colors of the Ocean Plastics. Environ Sci Technol 54, 6594-6601.

Mazzariol, S., Centelleghe, C., 2017. Standard protocol for post-mortem examination on cetaceans. Blue World Institute of Marine Research and Conservation..

Miller, M.E., Kroon, F.J., Motti, C.A., 2017. Recovering microplastics from marine samples: A review of current practices. Marine Pollution Bulletin 123, 6-18.

Nan, B., Su, L., Kellar, C., Craig, N.J., Keough, M.J., Pettigrove, V., 2020. Identification of microplastics in surface water and Australian freshwater shrimp Paratya australiensis in Victoria, Australia. Environmental Pollution 259, 113865.

National Oceanic and Atmospheric Administration, 2020. NOAA Evaluates Using Mussel Watch Program to Monitor Microplastics.

Nelms, S.E., Barnett, J., Brownlow, A., Davison, N.J., Deaville, R., Galloway, T.S., Lindeque, P.K., Santillo, D., Godley, B.J., 2019a. Microplastics in marine mammals stranded around the British coast: ubiquitous but transitory? Scientific Reports 9, 1075.

Nelms, S.E., Parry, H.E., Bennett, K.A., Galloway, T.S., Godley, B.J., Santillo, D., Lindeque, P.K., 2019b. What goes in, must come out: Combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator. Methods in Ecology and Evolution 10, 1712-1722.

Ogunola, S.O., Reis-Santos, P., Wootton, N., Gillanders, B.M., 2023. Investigating microplastic contamination and biomagnification in a remote area of South Australia. Marine and Freshwater Research 74, 917-927.

Ortega-Borchardt, J.Á., Ramírez-Álvarez, N., Rios Mendoza, L.M., Gallo-Reynoso, J.P., Barba-Acuña, I.D., García-Hernández, J., Égido-Villarreal, J., Kubenik, T., 2023. Detection of microplastic particles in scats from different colonies of California sea lions (*Zalophus californianus*) in the Gulf of California, Mexico: A preliminary study. Marine Pollution Bulletin 186, 114433.

Perera, K., Ziajahromi, S., Bengtson Nash, S., Manage, P.M., Leusch, F.D.L., 2022. Airborne Microplastics in Indoor and Outdoor Environments of a Developing Country in South Asia: Abundance, Distribution, Morphology, and Possible Sources. Environmental Science & Technology 56, 16676-16685.

Pfeiffer, F., Fischer, E.K., 2020. Various digestion protocols within microplastic sample processing—evaluating the resistance of different synthetic polymers and the efficiency of biogenic organic matter destruction. Frontiers in Environmental Science 8.

Phan, S., Padilla-Gamiño, J.L., Luscombe, C.K., 2022. The effect of weathering environments on microplastic chemical identification with Raman and IR spectroscopy: Part I. polyethylene and polypropylene. Polymer Testing 116, 107752.

Piperagkas, O., Papageorgiou, N., Karakassis, I., 2019. Qualitative and quantitative assessment of microplastics in three sandy Mediterranean beaches, including different methodological approaches. Estuarine, Coastal and Shelf Science 219, 169-175.

Plön, S., de Wet, M., Lane, E., Wohlsein, P., Siebert, U., Thompson, P., 2015. A standardized necropsy protocol for health investigations of small cetaceans in southern Africa. African Journal of Wildlife Research 45, 332-341.

Provencher, J.F., Ammendolia, J., Rochman, C.M., Mallory, M.L., 2019a. Assessing plastic debris in aquatic food webs: what we know and don't know about uptake and trophic transfer. Environmental Reviews 27, 304-317.

Provencher, J.F., Bond, A.L., Avery-Gomm, S., Borrelle, S.B., Rebolledo, E.L.B., Hammer, S., Kuhn, S., Lavers, J.L., Mallory, M.L., Trevail, A., van Franeker, J.A., 2017. Quantifying ingested debris in marine megafauna: a review and recommendations for standardization. Analytical Methods 9, 1454-1469.

Provencher, J.F., Borrelle, S.B., Bond, A.L., Lavers, J.L., Franeker, J.A.v., Kühn, S., Hammer, S., Avery-Gomm, S., Mallory, M.L., 2019b. Recommended best practices for plastic and litter ingestion studies in marine birds: Collection, processing, and reporting. FACETS 4, 111-130.

Provenza, F., Rampih, D., Pignattelli, S., Pastorino, P., Barceló, D., Prearo, M., Specchiulli, A., Renzi, M., 2022. Mussel watch program for microplastics in the Mediterranean sea: Identification of biomarkers of exposure using *Mytilus galloprovincialis*. Ecological Indicators 142, 109212.

Przeslawski, R., Barrett, N., Carroll, A., Foster, S., Gibbons, B., Jordan, A., Monk, J., Langlois, T., Lara-Lopez, A., Pearlman, J., Picard, K., Pini-Fitzsimmons, J., van Ruth, P., Williams, J., 2023. Developing an ocean best practice: A case study of marine sampling practices from Australia. Frontiers in Marine Science 10.

Quinn, G.P., Keough, M.J., 2002. Experimental Design and Data Analysis for Biologists.

Rani, M., Ducoli, S., Depero, L.E., Prica, M., Tubić, A., Ademovic, Z., Morrison, L., Federici, S., 2023. A complete guide to extraction methods of microplastics from complex environmental matrices. Molecules 28.

Rodrigues, M., Abrantes, N., Gonçalves, F., Nogueira, H., Marques, J., Gonçalves, A., 2018. Spatial and temporal distribution of microplastics in water and sediments of a freshwater system (Antuã River, Portugal). Science of the Total Environment 633, 1549-1559.

Samandra, S., Singh, J., Plaisted, K., Mescall, O.J., Symons, B., Xie, S., Ellis, A.V., Clarke, B.O., 2023. Quantifying environmental emissions of microplastics from urban rivers in Melbourne, Australia. Marine Pollution Bulletin 189, 114709.

Santana, M., Kroon, F.J., van Herwerden, L., Vamvounis, G., Motti, C.A., 2022. An assessment workflow to recover microplastics from complex biological matrices. Marine Pollution Bulletin 179, 113676.

Santos, R.G., Machovsky-Capuska, G.E., Andrades, R., 2021. Plastic ingestion as an evolutionary trap: Toward a holistic understanding. Science 373, 56-60.

Savoca, M.S., Kühn, S., Sun, C., Avery-Gomm, S., Choy, C.A., Dudas, S., Hong, S.H., Hyrenbach, K.D., Li, T.H., Ng, C.K., Provencher, J.F., Lynch, J.M., 2022. Towards a North Pacific Ocean long-term monitoring program for plastic pollution: A review and recommendations for plastic ingestion bioindicators. Environmental Pollution 310, 119861.

Schlawinsky, M., Santana, M.F.M., Motti, C.A., Martins, A.B., Thomas-Hall, P., Miller, M.E., Lefèvre, C., Kroon, F.J., 2022. Improved microplastic processing from complex biological samples using a customized vacuum filtration apparatus. Limnology and Oceanography: Methods 20, 553-567.

Schönlau, C., Karlsson, T.M., Rotander, A., Nilsson, H., Engwall, M., van Bavel, B., Kärrman, A., 2020. Microplastics in sea-surface waters surrounding Sweden sampled by manta trawl and in-situ pump. Marine Pollution Bulletin 153, 111019.

Skalska, K., Ockelford, A., Ebdon, J.E., Cundy, A.B., 2020. Riverine microplastics: Behaviour, spatio-temporal variability, and recommendations for standardised sampling and monitoring. Journal of Water Process Engineering 38, 101600.

Song, Y.K., Hong, S.H., Jang, M., Kang, J.-H., Kwon, O.Y., Han, G.M., Shim, W.J., 2014. Large accumulation of micro-sized synthetic polymer particles in the sea surface microlayer. Environmental Science & Technology 48, 9014-9021.

Tagg, A.S., Harrison, J.P., Ju-Nam, Y., Sapp, M., Bradley, E.L., Sinclair, C.J., Ojeda, J.J., 2017. Fenton's reagent for the rapid and efficient isolation of microplastics from wastewater. Chemical Communications 53, 372-375.

Valente, T., Ventura, D., Matiddi, M., Sbrana, A., Silvestri, C., Piermarini, R., Jacomini, C., Costantini, M.L., 2023. Image processing tools in the study of environmental contamination by microplastics: reliability and perspectives. Environmental Science and Pollution Research 30, 298-309.

Viršek, M.K., Palatinus, A., Koren, Š., Peterlin, M., Horvat, P., Kržan, A., 2016. Protocol for microplastics sampling on the sea surface and sample analysis. JoVE (Journal of Visualized Experiments), e55161.

Wessel, C.C., Lockridge, G.R., Battiste, D., Cebrian, J., 2016. Abundance and characteristics of microplastics in beach sediments: Insights into microplastic accumulation in northern Gulf of Mexico estuaries. Marine Pollution Bulletin 109, 178-183.

Wilkinson, M.D., Dumontier, M., Aalbersberg, I.J., Appleton, G., Axton, M., Baak, A., Blomberg, N., Boiten, J.-W., da Silva Santos, L.B., Bourne, P.E., Bouwman, J., Brookes, A.J., Clark, T., Crosas, M., Dillo, I., Dumon, O., Edmunds, S., Evelo, C.T., Finkers, R., Gonzalez-Beltran, A., Gray, A.J.G., Groth, P., Goble, C., Grethe, J.S., Heringa, J., 't Hoen, P.A.C., Hooft, R., Kuhn, T., Kok, R., Kok, J., Lusher, S.J., Martone, M.E., Mons, A., Packer, A.L., Persson, B., Rocca-Serra, P., Roos, M., van Schaik, R., Sansone, S.-A., Schultes, E., Sengstag, T., Slater, T., Strawn, G., Swertz, M.A., Thompson, M., van der Lei, J., van Mulligen, E., Velterop, J., Waagmeester, A., Wittenburg, P., Wolstencroft, K., Zhao, J., Mons, B., 2016. The FAIR Guiding Principles for scientific data management and stewardship. Scientific Data 3, 160018.

Wootton, N., Reis-Santos, P., Gillanders, B.M., 2021. Microplastic in fish – A global synthesis. Reviews in Fish Biology and Fisheries 31, 753-771.

Yap, V.H.S., Chase, Z., Wright, J.T., Hurd, C.L., Lavers, J.L., Lenz, M., 2020. A comparison with natural particles reveals a small specific effect of PVC microplastics on mussel performance. Marine Pollution Bulletin 160, 111703.

Zantis, L.J., Carroll, E.L., Nelms, S.E., Bosker, T., 2021. Marine mammals and microplastics: A systematic review and call for standardisation. Environmental Pollution 269, 116142.

Zhu, X., 2015. Optimization of elutriation device for filtration of microplastic particles from sediment. Marine Pollution Bulletin 92, 69-72.