Standardization of Operating Procedures for the Collection of Samples for Microplastics Analysis Part 2: Ambient Water

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SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT

Technical Report 1410.B

Standardization of Operating Procedures for the Collection of Samples for Microplastics Analysis
Part 2: Ambient Water

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EXECUTIVE SUMMARY

The California Statewide Microplastics Strategy and the California Statewide Plastics Monitoring Strategy both describe a need and proposed approach to gather microplastic monitoring data to inform management decisions. The development and use of harmonized methods for sample collection and analysis are critical parts of the proposed monitoring plan to ensure that comparable and reliable datasets are generated. Analytical methods for measuring and characterizing microplastics have already been evaluated and harmonized for a range of matrices including drinking water, environmental water, wastewater effluent, biological tissues, and sediment. Sample collection methods have been developed for some matrices but generally continue to be highly variable and lack harmonization. Therefore, the goal of this project was to produce harmonized collection methods for surface sediments, aquatic biota, ambient water, and stormwater flows. For each matrix, an expert working group was tasked with creating a protocol for standardization, developing evaluation studies as needed. This report provides a standard sample collection protocol for ambient water based on monitoring questions and goals. Guidance for quality control and the mitigation and characterization of background contamination (i.e., microplastic particles inadvertently introduced during sample collection) is also provided. The protocol also underwent field testing to determine the potential impact of variations in sampling device (pump [gear, peristaltic, and submersible] vs. trawl), pump tubing inlet diameter (6.3 vs. 9.5 mm), and sample volume (10, 20, 40, 80, vs. 160 L). This document is the second installment in a larger series of microplastics sampling protocols, following sediment and biota (Part 1) and preceding stormwater flows (Part 3).

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Background

The California Statewide Microplastics Strategy outlines a plan to create a monitoring network by leveraging existing monitoring programs throughout the state. Successful network implementation will require harmonized methods for sample collection, processing, analysis, and data management to generate useful, high-quality results to inform management decisions. Sample processing and analysis methods have already been developed and evaluated for multiple matrices (De Frond et al., 2022, Thornton Hampton et al., 2023). Less work has been done for sample collection, but some methods have recently been made available for aqueous matrices, including ambient water. The American Society for Testing and Materials (ASTM) has published guidance on the collection of water samples with low to high suspended solids for microplastics analysis where water is passed through a series of sieves to capture and concentrate microplastic particles (ASTM D8332). The International Organization for Standardization (ISO) also provides guidance on this type of approach, which it refers to as cascade filtration, as well as the collection of grab samples and net samples (ISO 5667-27:2025). The availability of these guidance documents and protocols represents an important first step toward method harmonization for microplastics monitoring in water.

No single collection method can capture the entire diversity of microplastics in water. Existing approaches include a variety of methods, each targeting a different subpopulation of microplastics (Brander et al., 2020). Each method also comes with unique advantages and disadvantages. Net sampling, the most common sampling method, captures microplastics floating on the surface but can also be used to collect samples at various water column depths. Large volumes of water can be sampled over an extensive sampling area, specifically in the case of towed nets. One potential drawback is that particles smaller than the net mesh size (often ~330 µm) are excluded. To capture particles below the surface, water may be pumped through a series of sieves or filters to collect microplastics at discrete depths or depth-integrated across the water column. For both pump and net sampling, the minimum particle size captured is determined by the smallest sieve mesh or filter pore size used; therefore, both methods can capture smaller particles when finer mesh or filters are used. However, clogging of the sieves, mesh, or filters can impose limitations on the volume and shift the minimum particle size collected, particularly when water contains elevated levels of suspended solids. Grab samples involve collecting a known volume of water directly into a container. While this approach is limited to small volumes, which may not be representative of the habitat or area of interest (e.g., ~1-20 L), it can capture particles that may otherwise pass through nets, sieves, or filters, including fibers and nanoplastics. The variety of microplastic collection methods available for environmental water provide flexibility, but methods must be selected carefully (including

sampling the appropriate volume) to ensure that it targets the appropriate subpopulation of microplastic particles and addresses monitoring goals within logistical and cost constraints.

To address the specific needs of the California Statewide Microplastics Strategy and advance collection method harmonization and standardization for microplastics, an expert working group was convened to identify and evaluate methods for ambient water to create standard operating procedures for the collection of ambient water for microplastics analysis. Experts developed the collection protocol provided in this document with options for both net and pump sampling. The protocol was field-tested in a semi-controlled experiment to ensure robustness and evaluate key variables identified by experts that would potentially have strong influences on method performance and results. This study methods and results are briefly summarized in Appendix A but are described in detail elsewhere (Li Ludeña et al., *In Preparation*). This project is part of a larger effort to identify and evaluate appropriate collection methods for sediment, biota, environmental water, and stormwater flows. Collection methods for sediment and biota were addressed in a previous report (Part 1, Thornton Hampton et al., 2025), and collection methods for stormwater flows will be addressed in a future report (Part 3).

STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF WATER SAMPLES FOR MICROPLASTICS ANALYSIS

1.0 Scope and Application

1.1 This method is for the collection of freshwater, brackish water, or seawater samples for the determination of the concentration and composition of microplastics, as defined by the State Water Resources Control Board (2020). The method is based on peer-reviewed literature and recommendations from an international expert panel and working group convened and coordinated by the Southern California Coastal Water Research Project Authority. This method summarizes current best practices and more detailed guidance (e.g., sampling volumes, sampling devices, and approach) may continue to evolve through further study. This sampling protocol is intended to provide guidance for research purposes as well as the development of microplastic monitoring programs.

1.2 Sample processing and analysis methods are not within the scope of this method. Standardized procedures for the extraction and measurement of microplastic particles in drinking water are available and have been adopted by the State Water Resources Control Board (State Water Resources Control Board 2022a, 2022b). Procedures for the extraction and measurement of microplastic particles in water have been tested and described in other related work (Thornton-Hampton et al., 2023).

1.3 Microplastics are ubiquitous environmental contaminants. It is impossible to eliminate background contamination from airborne particles, plastic or otherwise. This method provides recommendations for mitigating and characterizing background contamination during sample collection.

1.4 Procedural requirements can be distinguished from guidance elements based on the language used. Specifically, the use of MUST, SHALL, and command statements, such as “clean all equipment thoroughly before use,” indicate procedural requirements, whereas non-commanding language such as SHOULD indicates guidance.

1.5 Study leads are responsible for obtaining permits prior to sampling. If sampling on private property, permission from the landowner must be obtained. Note that obtaining multiple permits may be necessary to fulfill private, State, and Federal requirements. See Moulton II et al. (2002), for additional guidance.

2.0 Summary of Method

This method describes the environmental sampling of microplastics from water in lakes, bays, estuaries, and oceans. A separate protocol will be developed for sampling rivers and stormwater as different techniques are used in flowing water. This method captures microplastics and other solid material via pumping bulk water through filters or sieves and offers a complementary method for surface water sampling with a surface trawl net where needed. These two methods (i.e., pumps and surface trawls) were selected because they are commonly used water sampling techniques for microplastics. Monitoring objectives will vary, and thus specific aspects of this standard operating procedure may be modified where indicated. Based on the monitoring objectives (e.g., concentrations over time, space, etc., as well as interest in depth), users can select details of sampling (e.g., filter/sieve size, water volume, pump type, sampling depth, whether to include surface trawls, etc.). For example, the choice of pump is dependent on sampling and logistical conditions (e.g., pump rate, target depth, power supply, and cost). Additionally, tubing material and diameter is dependent on pump type and the largest size of particles that will be collected. A minimum particle size of 50 µm is recommended as analytical methods have not yet been evaluated below this size range (State Water Resources Control Board 2022a, 2022b; Thornton Hampton et al., 2023) See Table 1 below for more guidance.

Table 1. Summary of sampling method decisions associated with sampling via pump and surface trawl.

Method	Decision
Pump	**Type of Pump**: (e.g., peristaltic, gear, submersible). Different pumps may be more feasible depending on sampling depth and location. Test pump for contamination and recovery.
	Tubing: Use silicon or Tygon tubing. The inner diameter should be 6-15mm and at least three times the size of the largest particle of interest.
	Size Fractionation: Use 50 µm as the smallest mesh size and pump the sample through a sieve stack or in-line filtration unit with at least one larger size fraction on top to help reduce clogging.
	Sample Volume: At minimum, the volume should be enough to ensure that the amount of predicted microplastic particles is greater than the minimum detectable amount (which is determined by background contamination levels). Specific minimum particle counts are also dependent on monitoring goals.
	Depth of Sampling: Take a depth-integrated sample from just below the surface to one meter depth. Sampling can also be taken from other depths to meet story and/or monitoring objectives (e.g., discrete depths to sample a depth profile).
Surface Trawl To be used in addition to pump sampling if there is interest in sampling the air-water interface	**Type of Trawl**: Use a manta trawl with a calibrated flow meter, but any surface trawl would work for this purpose as long as there is a method to measure the volume sampled. Test your trawl for contamination and recovery.
	Size of Mesh: Use a mesh size that matches one of the size fractions that overlaps with pump sampling (e.g., if one of the sieves in the stack is 100 µm, a 100 µm mesh size for the trawl would be appropriate).

Method	Decision
	Sampling Time: The sampling time along with the net intake area, and net intake velocity will determine the sample volume. The volume should be enough to ensure that the amount of microplastics in the samples is greater than the minimum detectable amount (which is determined by background contamination levels) without overloading the net and that the coefficient of variability between duplicate samples is < 30%. Specific minimum particle counts are also dependent on monitoring goals.

3.0 Definitions

Field blank (FB) – An aliquot of MAG water (Microplastics Analysis Grade, see definition below) that is placed in a clean container in the laboratory before going into the field, and then treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, running through the sampling procedure, storage, preservation, and all analytical procedures. The volume of the FB must be similar to that of actual samples collected and processed by this method. A wetted filter placed in a clean glass petri dish may also be used. If using a wetted filter, the pore size should be less than the lower particle size limit of the study. The purpose of the FB is to determine if method analytes or other interferences are introduced into the samples during shipment and collection. At least one FB must be collected for each sampling event (see definition below). FBs differ from Trip Blanks (see definition below) in that the FB evaluates contamination during both shipment and collection, while the Trip Blank only accounts for contamination during shipment. Given that the FB is analyzed alongside field collected samples, the FB may also serve as the Laboratory Reagent Blank (LRB). If this approach is used, it is recommended that a subset of LRBs is analyzed separately to identify potential sources of contamination. For more details regarding the LRB, please see "Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water" and "Standard Operating Procedures for Extraction and Measurement by Raman Spectroscopy of Microplastic Particles in Drinking Water" (State Water Resources Control Board, 2022a, 2022b; Lao and Wong, 2023). If the FB plastic particle count is greater than the Minimum Reporting Level (see definition below), all associated field collected samples (i.e., collected during the same sampling event) must be flagged during data reporting.

For water samples, collection of FB should happen independently for each sampling method (i.e., pump and surface trawl if both are used) unless deployed at the same time. The volume of each FB will ideally be the same as the sample volume, but this is not always feasible. Thus, the FB should be at least 4L. FBs must be analyzed alongside field collected samples from the same sampling event.

Microplastics – Solid[1] polymeric materials[2] to which man-made chemical additives or other substances may have been added. Particles must have at least three dimensions that are greater than 1 nm and less than 5,000 µm. Polymers that are derived in nature that have not been chemically modified (other than by hydrolysis) are excluded (State Water Resources Control Board, 2020).

Microplastics-analysis-grade (MAG) water – Water filtered through a pore-size of 1µm or smaller. Filters must be of an appropriate material that does not shed plastic particles (Section 6). MAG water is used as reagent water and to rinse apparatus. Appropriate sources of water to generate MAG water are deionized water, reverse osmosis water, or 18 MΩ cm water (e.g., nanopure/MilliQ water).

Minimum reporting level (MRL) – The minimum concentration that can be reported by a laboratory as a quantified value in a sample following analysis. See "Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water" and "Standard Operating Procedures for Extraction and Measurement by Raman Spectroscopy of Microplastic Particles in Drinking Water" for more details (State Water Resources Control Board, 2022a, 2022b; Lao and Wong, 2023).

Sampling event – Sample collection that occurs within a 24-hour period at the same site with the same field crew using a given sampling method and sampling device.

Trip blank – A sample of MAG water of a similar volume as test samples, taken from the laboratory to the sampling site and returned without having been exposed to sampling

[1] 'Solid' means a substance or mixture which does not meet the definitions of liquid or gas. 'Liquid' means a substance or mixture which (i) at 50 degrees Celsius (˚C) has a vapor pressure less than or equal to 300 kPa; (ii) is not completely gaseous at 20 ˚C and at a standard pressure of 101.3 kPa; and (iii) which has a melting point or initial melting point of 20 ˚C or less at a standard pressure of 101.3 kPa.
'Gas' means a substance which (i) at 50 ˚C has a vapor pressure greater than 300 kPa (absolute); or (ii) is completely gaseous at 20 ˚C at a standard pressure of 101.3 kPa.

[2] 'Polymeric material' means either (i) a particle of any composition with a continuous polymer surface coating of any thickness, or (ii) a particle of any composition with a polymer content of greater than or equal to 1% by mass. 'Particle' means a minute piece of matter with defined physical boundaries; a defined physical boundary is an interface. 'Polymer' means a substance consisting of molecules characterized by the sequence of one or more types of monomer units. Such molecules must be distributed over a range of molecular weights wherein differences in the molecular weight are primarily attributable to differences in the number of monomer units. A polymer comprises the following: (a) a simple weight majority of molecules containing at least three monomer units which are covalently bound to at least one other monomer unit or other reactant; (b) less than a simple weight majority of molecules of the same molecular weight. 'Monomer unit' means the reacted form of a monomer substance in a polymer. 'Monomer' means a substance which is capable of forming covalent bonds with a sequence of additional like or unlike molecules under the conditions of the relevant polymer-forming reaction used for the particular process.

procedures and the environment outside of the lab. The trip blank is to assess contamination introduced during shipping and storage only and must be present for each set of field samples from a sampling event. A trip blank must be evaluated for shedding (Section 4.2) prior to the sampling event if different sampling containers from those listed in Section 6 are used. If total plastic particle counts are greater than the MRL, the sampling container may not be used. A trip blank is only analyzed if microplastic particle counts in the FB exceed the MRL and contamination is suspected to have occurred during transport.

4.0 Interferences

4.1 Physical Interferences

4.1.1 Preventing ambient water samples from becoming contaminated during collection can be one of the greatest difficulties encountered in quantifying microplastics in water samples. It is not possible to confidently eliminate all contamination from samples during collection, however quantifying the contamination is possible. It is important that extreme care be taken to minimize contamination when collecting water samples for microplastics. Controlling particle contamination requires strict adherence to protocols for contamination control as outlined in Section 4.2.

4.1.2 Major sources of particle contamination in the field during sample collection include, but are not limited to: fibers from clothing and textiles (including lab coats, particles from any equipment made of plastic, synthetic ropes and lines on sampling vessels, apparel worn by field personnel, carpets, and furniture), chipping paint on sampling vessels, particles deposited from the air, particles settled on equipment prior to use, non-MAG water, water used to clean equipment prior to use, sponges or brushes used to clean equipment prior to use, inadequate cleaning of sampling equipment, synthetic polymer gloves, and plastic sample containers and lids.

4.2 Contamination Control

4.2.1 Irrespective of the requirements in Section 4.2 and elsewhere in this SOP, the safety of field crews is paramount and takes precedence over all other considerations.

4.2.2 Field crews and laboratory personnel should use as much plastic-free equipment and consumables as possible, except where allowed as noted in Sections 4.2.2.3 to 4.2.2.7.

4.2.2.1 Field crews and laboratory personnel must use equipment throughout the process composed of glass (e.g., sampling jars) or metal (e.g., foil, sampling devices, scoops), except as noted in Sections 4.2.2.3 to 4.2.2.8.

4.2.2.2 All materials used for cleaning of equipment prior to use must be made of natural/non-plastic materials (e.g., natural-based material sponge, horsehair brush).

4.2.2.3 Use of plastic tubing (e.g., Tygon®, silicone) to dispense water used to make MAG water is acceptable. Minimal contamination has been attributed to these materials.

4.2.2.4 Typical laboratory-grade solvent squeeze bottles (e.g., Teflon or polyethylene) are also suitable to dispense MAG water for the rinsing of sampling devices, sampling jars, and other equipment as long as they are used similarly for QA/QC samples (e.g., FBs and Trip Blanks). Minimal contamination has been attributed to these sources.

4.2.2.5 Purple nitrile gloves (e.g., Kimtech®) have minimal contamination potential and their purple color allows field crews and laboratory personnel to easily distinguish if any contamination may be attributed to them.

4.2.2.6 The pump should have tubing made of Tygon® or silicone (see Section 4.2.2.3). These materials have minimal contamination potential but should be thoroughly cleaned, stored in a dark place, and evaluated for shedding according to procedures described in Section 8 – Quality Control.

4.2.2.7 Nets used for surface trawls are often made from plastic, typically nylon. Any netting used for trawling must be thoroughly inspected for their integrity and evaluated for shedding according to procedures described in Section 8 – Quality Control. It is also recommended that a sample of the netting material be taken to compare to particles found in samples.

4.2.2.8 If plastic materials are used, inspect their integrity. For example, potential contamination from sampling jars and lids should be tested before choosing them to be used (Section 8.3). Once a jar or lid is picked for your monitoring program, do not deviate from it unless new materials are tested. FBs exist to help account for any procedural contamination from plastics during sample collection. Examples of plastics commonly used in microplastics analysis that are acceptable as they do not shed polymer particles are listed in Section 6.0 – Equipment and Supplies.

4.2.2.9 All plastic apparatus shall be evaluated for potential to shed microplastics biannually using the procedures noted in Section 8 – Quality Control.

4.2.3 Ensure a clean working environment before and during sample collection.

4.2.3.1 Inspect sampling gear and equipment onboard boats or areas near the sampling location for plastic debris or other potential sources of particle contamination. Remove or replace materials as necessary. For items that cannot be removed or replaced that may contribute to contamination, it is recommended to photograph and document their properties.

4.2.4 Minimize the use of synthetic textiles in the field.

4.2.4.1 It is recommended that field crews do not wear synthetic clothing when collecting samples. If possible, wear cotton laboratory coats or similar garments providing equivalent coverage (e.g., large, bright colored, lightweight cotton t-shirts), ideally of a noticeable color not commonly found in environmental samples (e.g., pink) to allow clear identification within samples as contamination. Exceptions may be made for extreme weather conditions (e.g., cold) though it is recommended that field crews document the type of clothing worn by taking photographs, taking detailed notes, and collecting textile samples to compare to particles found in samples.

4.2.4.2 If synthetic Personal Protective Equipment (PPE) must be worn for safety reasons, it is recommended that field crews document the type of clothing worn by taking photographs, taking detailed notes, and collecting textile samples to compare to particles found in samples.

4.2.5 Clean all equipment thoroughly before use.

4.2.5.1 Before each sampling event, wash all glassware and tools with low-foam soap and hot water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water and then three times with MAG water.

4.2.5.2 Heavy-duty aluminum foil can be used to cover cleaned apparatuses and tools such as forceps to protect from airborne particulate contamination. Foil may be pre-ashed at ≥ 450 °C for at least 1 hour before use to destroy all organic material, then stored in a covered non-plastic container. Discard foil after use.

4.2.5.3 During each sampling event, rinse all glassware and tools with MAG water between each sample collected.

4.2.5.4 When feasible, cover all equipment with pre-ashed, heavy-duty aluminum foil or clean glass when not in use in the field or when stored.

4.2.5.5 Pressurized air can be used to remove possible contamination on the surface of equipment prior to use. If compressed gas is used to blow-dry equipment or samples for microplastics, ensure that the air is clean (e.g., put a 1 µm metal filter between the source and the outlet).

5.0 Safety

5.1 The safety of field crews is the first priority of any field collection activity and supersedes all requirements and recommendations in this document.

5.2 Field crews must be aware of all safety procedures and potential hazards associated with each sampling event and sampling site. Each field crew must follow all established rules and provisions within their respective organization's safety program.

5.3 No analytes or reagents of concern are used within this method.

5.4 Nitrile gloves (e.g. purple Kimtech®) are required for this method to minimize contamination from analysts.

6.0 Sample Size and Material Amount Recommendations

The target volume of water collected will vary based on the specific monitoring goals and sampling scenario but should aim to be representative of the sampling environment. A target sample volume may be selected based on 1) the target minimum number of particles to be captured and 2) the predicted range of microplastic concentrations in the sampling environment.

The target minimum number of particles to be captured may be selected based on specific monitoring goals. However, the minimum number of particles captured should aim to be at least above the Method Reporting Limit (calculated using the Minimum Detectable Amount (MDA) based on blank levels; see Lao and Wong, 2023). This value is driven by the level of procedural contamination during sampling and analysis, as quantified and characterized by the analysis of field and laboratory blanks, respectively. The determination of volume amounts to be collected and analyzed may be facilitated by using the supplementary MDA and Sampling Unit Calculator. This tool may be used to calculate MDAs and estimate the volume required for analysis to ensure detectable amounts of microplastics given an estimated level of background contamination.

The predicted range of microplastic concentrations may be estimated by investigating previously published microplastics data sets in comparable habitats or conducting a small pilot study. The pilot study may also be used to estimate background contamination levels for the calculation of the MDA as described previously.

Together, the target minimum number of particles to be captured and the predicted microplastic concentrations may be used to estimate a target minimum sample volume using the Representative Sample Volume Predictor (RSVP) tool (Cross et al., 2025). For example, researchers may determine that blank samples during sampling and analysis contain 10 ± 2 particles (mean ± standard deviation), which results in an MDA value of 22 particles. Given this, the minimum target number of particles to capture may be set at 50 in case higher background contamination occurs. The predicted minimum microplastic concentration is ~2 particles/L. Using the RSVP tool, a minimum sample volume of 30-37 L is estimated based on the desired level of statistical significance (e.g., $\alpha = 0.05$).

7.0 Equipment and Supplies

7.1 Equipment and supplies are listed in the table below. References to specific brands or catalog numbers are included as examples only and do not imply endorsement of the product. Such references do not preclude the use of other vendors or suppliers.

Item	Suggested Materials
Equipment Cleaning and Field Preparation	
Low-foam dish soap	Alcojet, Fisher Catalog no. 16-000-110
Sponge made from natural materials	Loofah, cellulose, natural sponge Amazon “natural sea sponge, 6-7 in”
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
1 µm pore-size filters for making MAG water if needed (e.g., some laboratories have taps for RO)	47 mm diameter, Sterlitech Polycarbonate Track Etch (PCTE) membrane filters, Catalog no. PCT1047100
Sample Collection (Pump)	
Nitrile gloves	Kimtech® Purple Nitrile Exam Gloves (Product code #55083)
Heavy-duty aluminum foil	-
Laboratory labeling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Pump w/power source (may be a battery that needs to be brought on board and charged, note that voltage will determine pumping rate)	-
Bucket with volume markings or spigot at the bottom with totalizer attached to measure outflow volume – check precision and consider error in any error budget	-
Sampling jars (for stacked sieves) or glass petri dishes (for in-line filtration)	Sampling Jars: Target “Ball 16oz 12pk Glass Wide Mouth Mason Jar with Lid and Band” OR

Item	Suggested Materials
	16oz Glass Wide Mouth Sampling Jars, Environmental Sampling Supply (Part #0500-0015-QC) See Section 10.2 for sampling jar requirements Petri Dishes: VWR Catalog no. 75845-542
Stainless steel mesh sieves (for stacked sieves)	VWR Catalog No. 57334-594 (53 µm mesh size) VWR Catalog No. 57334-582 (150 µm mesh size) VWR Catalog No. 57334-574 (300 µm mesh size)
Tubing	Silicon or Tygon® (6 – 15mm ID)
Weights for the bottom of the tubing (metal)	-
In-line filter holders (for in-line filtration)	Dependent on diameter of filter
Filter mesh (sold in various materials; for in-line filtration; avoid common plastic types (e.g., PP); recommended - stainless steel or nylon)	50 µm, 150 µm, 300 µm (recommended)
Forceps (for in-line filtration)	-
MAG water	-
Bubble wrap	-
Cooler or heavy-duty storage container	-
99% Isopropyl Alcohol (pre-filtered through 1 µm pore size filter) or Sodium Benzoate	VWR Catalog No. 470301-468 VWR Catalog No. AAAA15946-30
Sample Collection (Surface Trawl)	
Low-shedding rope or cable for deployment	-
Nitrile gloves	Kimtech® Purple Nitrile Exam Gloves (Product code #55083)
Heavy-duty aluminum foil	-
Laboratory labeling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Surface trawl w/cod end	KC Denmark Research Equipment, Manta trawl net, 70 x 30 cm (No. 23.440, 23.490, or 23.500)
Flow meter	KC Denmark Research Equipment, Digital Flow Meter (No. 23.090)
Sampling jars (glass recommended with heavy duty foil barrier used between the lid and jar)	Sampling Jars: Target “Ball 16oz 12pk Glass Wide Mouth Mason Jar with Lid and Band” OR 16oz Glass Wide Mouth Sampling Jars, Environmental Sampling Supply (Part #0500-0015-QC) See Section 10.2 for sampling jar requirements
MAG water	-
Bubble wrap	-
Cooler or heavy-duty storage container	-
99% Isopropyl Alcohol (pre-filtered through 1 µm pore size filter) or Sodium Benzoate	VWR Catalog No. 470301-468 VWR Catalog No. AAAA15946-30

8.0 Reagents and Standards

8.1 MAG water is required throughout the cleaning and sampling process to ensure that equipment and sampling jars are free of particle contamination. The MAG water is to be collected and stored in a clean vessel (Section 4.2.5.1) and covered (Section 4.2.5.2) until use.

8.2 The Field Blank(s) and Trip Blank(s) should be created by the laboratory using MAG water and clean (Section 4.2.5.1) glass or metal containers.

9.0 Quality Controls

This section describes each quality control (QC) parameter, its required frequency and the performance criteria that must be met to satisfy the quality objectives. These QC requirements are considered the minimum acceptable QC criteria. Field crews and laboratories are encouraged to institute additional QC practices to meet their specific needs.

9.1 Field Blank (FB) – A FB (Section 3) must be included with each sampling event and analyzed to assess contamination during sample collection. Microplastic levels must be below the MRL; if not, the batch of samples associated with the FB must be flagged accordingly. Analysis results from the FB must be reported alongside analysis results from collected samples.

9.2 Trip Blank – A Trip Blank (Section 3) must be evaluated before the sampling event if a different type of sampling container is used from those listed in Section 6. Trip blanks do not need to be analyzed unless the FB shows evidence of contamination (i.e., microplastic levels in the FB are greater than the MRL) and contamination is suspected to have occurred during travel or shipment. The Trip Blank may be analyzed to determine if contamination occurred during shipping or travel.

9.3 Contamination Control Verification – It must be verified that plastic equipment that comes into contact with samples does not shed particles. Rinse each piece of equipment three times with MAG water, collecting the rinsate in a clean sampling jar (see Section 10.2). Cover the opening of the jar with pre-kilned heavy-duty foil and store according to Section 10.1. The sample may then be shipped (Section 10.2) to the analytical laboratory to test as a blank sample. If the particle count from the rinse is greater than the MRL, the equipment must not be used. This technique may also be utilized to identify potential sources of contamination in the laboratory. Conduct this verification biannually (Section 4.2.2.9).

9.4 Duplicates – One duplicate sample, taken back-to-back or in parallel with a real sample, must be taken every 10 samples as a measure of precision and accuracy. If less than 10 samples are collected, at least one duplicate sample must be taken.

9.5 Flow meter – The flow meter used to measure the volume of water filtered when sampling via a surface trawl should be periodically calibrated according to the manufacturer's instructions. Calibration activities and results should be documented.

10.0 Procedure

The following procedures assume that all equipment and supplies have been cleaned according to previously described requirements (see Section 4.2.5).

10.1 Pump Sampling

There are several types of pumps that can be used. For example, a peristaltic or gear pump (e.g., ISCO brand) can be used with a weighted tube that is deployed from the boat. Note that pumps have ratings for depth. Make sure to choose a pump that will reach the depth of interest. Alternatively, a submersible pump can be used. The tubing should be Tygon® or silicone and the inner diameter at least 3 times the size of the largest particle of interest. A diameter of 6 mm – 15 mm or ¼ - ¾ inch is recommended. For peristaltic pumps, tubing wall thickness may need to be increased depending on head height (i.e., vertical distance from the air/water interface to the pump location).

Samples can be taken at the depth of choice based on the monitoring question of interest. For example, a sub-surface sample can be taken by holding the tube 10 cm below the surface. Alternatively, a depth-integrated sample can be taken by moving the tube or pump up and down slowly and steadily from recorded depths. Moreover, stratified sampling can be conducted by taking a set volume from each of several depths to get a vertical profile. The end of the tube should be weighted to help it stay straight up and down in the water column. For ambient waters with tidal currents this may require alternative methods such as attaching to an anchored line or accounting for the effect of the deflection angle on the vertical depth of the tubing intake.

The sample volume needs to be enough to ensure that the amount of microplastics in the sample are at least above the MRL (calculated using MDAs based on blank levels; see Lao and Wong, 2023). The sampling volume will depend on the levels of microplastic pollution in the sampling regions and the procedural contamination. For example, in regions with low microplastic contamination, volumes of > 100 L may be needed. In regions highly contaminated with microplastics, volumes may only need to be ~20 L. It is recommended to start with a pilot sampling study to assess the volume needed to meet these criteria without overloading the filters or sieves with debris, which may lead to clogging. The supplementary MDA and Sampling Unit Calculator may be used to calculate MDAs and estimate sample volumes needed to exceed the MDA.

Microplastics may be collected either by pumping water through a sieve stack or a series of in-line mesh filters (Figure 1). Using a sieve stack may help reduce clogging, whereas using in-line filtration may help reduce sample contamination. When filtering using a sieve stack, it is

recommended to cover the top sieve with foil to reduce airborne contamination. If filtering using in-line filters, it is recommended to place the filters upstream of the pump to avoid potential contamination from the pump.

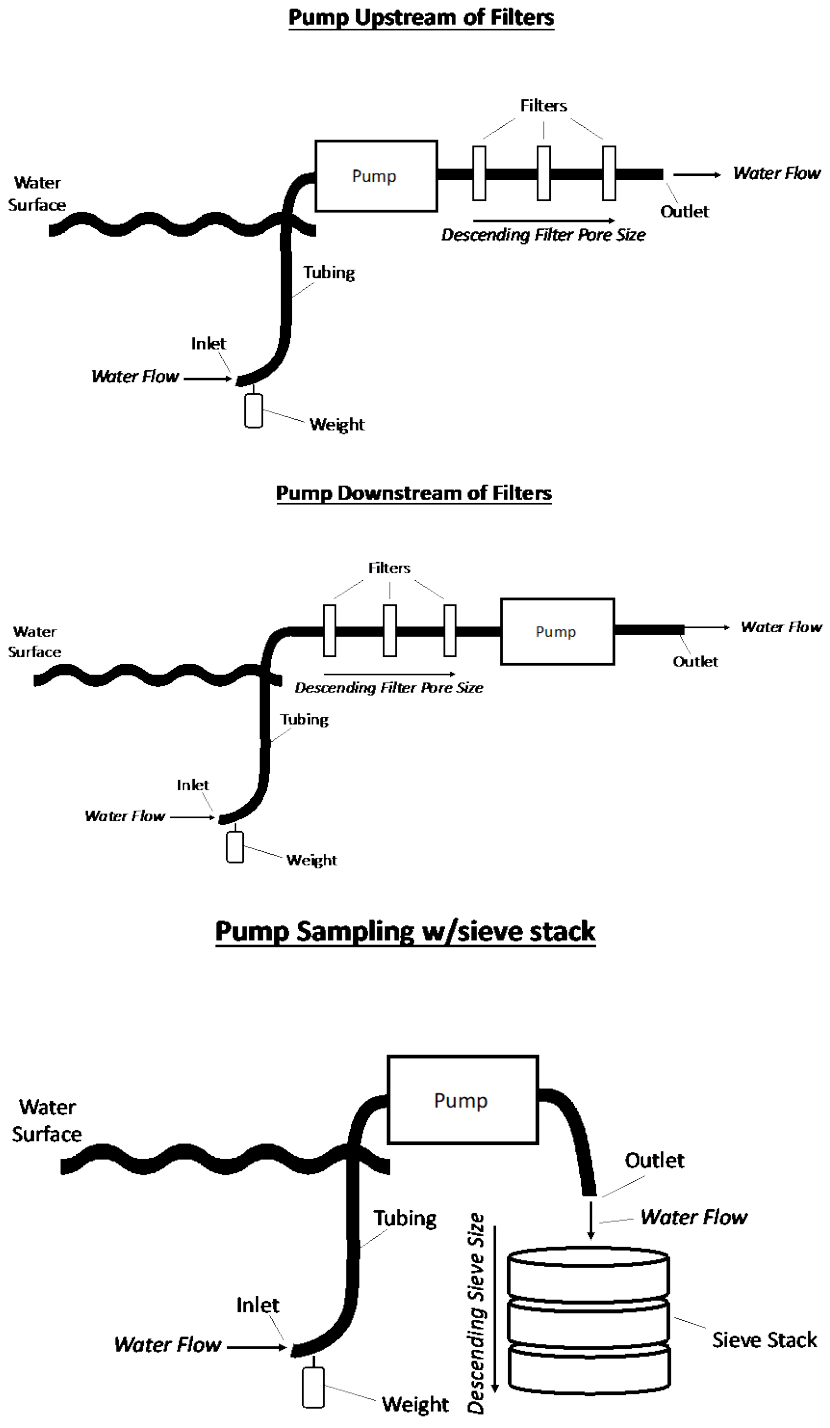

Figure 1. Basic diagram of sampling configurations where water is pumped up or downstream of filters or pumped through a sieve stack.

10.1.1 Record the sampling date, time, and location/GPS coordinates as well as the required weather, sea state, and water quality parameters detailed in Section 11.1.

10.1.2 Set up the pump and tubing so it is ready to deploy.

10.1.3 Purge the pump by running enough site water through the pump and tubing (and in-line filter holders if applicable) to reduce cross-contamination between sampling sites (e.g., 3x the volume of the tubing).

10.1.4 If using a sieve stack, stack the clean sieves with the smallest size on bottom and largest on top. For sieves or in-line filters, to keep track of volume collected, place the sieve stack on top of a marked bucket or the output tube inside the marked bucket. Another method would be to calibrate the pump to determine the pumping rate (L/min) or use a volume totalizer (flow meter) on the output tube. Note that the pump will likely slow down as filters clog and this should be noted, and potentially accounted for by changing filters or sieves and/or measuring the flow rate upon noticing any change in flow. If using in-line filtration, place the filters in the filter holders using forceps and attach the filter holders together in descending order according to pore size (i.e., water should flow through the filter with the largest pore size first and the smallest pore size last).

10.1.5 Position the tubing for sampling and turn on the pump, pumping water through the sieve stack or in-line filters until you reach the desired volume. Record the depth sampled, the time it takes to pump the sample, and the volume of water filtered.

10.1.6 If using a sieve stack, rinse each sieve with MAG water into a clean and labeled sample jar. If using in-line filtration, use clean tweezers to remove each filter, placing them in labeled clean glass petri dishes.

10.1.7 If using a sieve stack, add 0.1% sodium benzoate or 99% isopropyl alcohol to a volume of 10% of the sample to each sample jar. If using in-line filtration, and you are interested in storing the filter wet, it can be placed in MAG water with alcohol as well (choose an alcohol that is compatible with your filter material).

10.1.8 Label both the sample and FB jars, wrap in bubble wrap, and store in a secondary container for protection during transport.

10.2 Sampling via a surface trawl

This protocol recommends that a monitoring program sample using pumps; however, there may be a situation where a surface sample is preferred (e.g., studies show that some particles, e.g., foam, are not captured using pumps because they miss the air-sea interface) or net

sampling is needed to acquire enough volume to get a representative sample. If your objectives require a surface or net sample, follow this protocol. It is recommended to start with a pilot sampling study to assess the volume needed to meet these criteria without overloading the trawl net, which may lead to clogging and challenges during sample processing and analysis. The supplementary MDA and Sampling Unit Calculator may be used to calculate MDAs and estimate sample volumes needed to exceed the MDA.

Sampling via a surface trawl is best conducted under calm surface conditions. Ideally, sampling should only occur when wave heights are under 0.5 m and the Beaufort wind force scale is under 3 as well as avoiding conditions including high densities of natural particles or organisms (e.g., Michida et al., 2023).

10.2.1 Record the sampling date, time, and location as well as the required weather, sea conditions, and water quality parameters detailed in Section 11.1. Record the GPS coordinates of your starting position.

10.2.2 Dip the net with the cod end removed in site water to reduce any cross-contamination. The cod end can also be rinsed in site water.

10.2.3 Attach the flow meter to the net, reattach the cod end, and set up the net to be deployed on the side of the boat (outside the wake). Record the net position (side or stern of vessel) and distance from the vessel.

10.2.4 Record the flow meter and start time, deploy the net, and drive the boat 1-2 knots for the sampling period (e.g., 15 min). Record the trawl direction and vessel speed.

10.2.5 When removing the net, record the end time, flow meter reading, and GPS immediately.

10.2.6 Wash contents into the cod end using site water. Wash from the outside of the net to minimize background contamination.

10.2.7 Empty remaining contents of the cod end into a clean and labeled sample jar using MAG water.

10.2.8 Add 10% by volume 99% isopropyl alcohol or 0.1% sodium benzoate to the sample.

10.2.9 Label both the sample and FB jars, wrap in bubble wrap, and store in a secondary container for protection during transport.

11.0 Sample Preservation and Storage

11.1 Samples must be stored at 4°C to prevent growth of biotic material. Samples may be preserved by adding 99% isopropyl alcohol (10% by volume) sodium benzoate (final concentration of 0.1% mass per volume). Samples must also be kept away from direct sunlight or bright light. Samples that must be stored near direct sunlight or bright light should be stored in opaque containers or containers covered by aluminum foil.

11.2 Glass containers with non-plastic lid liners (although PTFE is acceptable), pre-cleaned as with other apparatuses (Section 4.2.5) in this method, must be used to collect and store samples to minimize microplastic contamination from the container when feasible. If there is concern regarding breakage during storage, travel, or shipment, other containers may be used as long as they are evaluated for shedding (Section 8.3). Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts or other packing materials that may easily fragment if possible; if not, then ensure that containers are sealed prior to shipment and the outsides are rinsed thoroughly before the sample is opened to prevent contamination.

11.3 Trip Blanks should accompany sample bottles to the sampling site and back to the laboratory after sample collection. Do not open Trip Blanks in the field; Trip Blanks must remain sealed until analysis. Trip Blanks may be used to identify potential sources of contamination occurring from shipping the sample container to the site and back, and do not need to be analyzed unless evidence of contamination during shipment arises from analysis of FBs.

11.4 Field Blanks must accompany each set of sample containers taken from the laboratory to the sampling site and back. The FB should be run through the sampling method, using the same methods used for sampling as best as possible (e.g., running the field blank water through the pump), and “collected” during the sampling campaign. It should be stored the same way as real samples. At least one FB must be transported and analyzed for each sampling event.

12.0 Field Data Reporting Requirements

12.1 Data to be reported when sampling ambient water for microplastics are listed in the table below.

Sampling data and location	Sampling date, start time, and end time	
	Sampling location	
Sampling equipment	*Sampling via a pump*	Type and brand of pump
		Type and size of tubing (ID)
		Size fractions and material of sieves or mesh filters and holders
	Sampling via a surface trawl	Type of net and frame/net dimensions (width, height, and length)
		Size fractions and material of sieves
Pumping or trawling parameters	*Sampling via a pump*	Pump speed or pump duration
		Filtered water volume
		Depth sampled
	Sampling via a surface trawl	GPS coordinates at the start and end of the trawl
		Trawl distance
		Trawl duration
		Trawl direction
		Vessel speed
		Net position and distance from vessel
		Filtered water volume
Quality Control	Field Blank Volume	Volume used for field blank
	Replicate Number	Number of replicates collected
Metadata (e.g., weather, sea conditions, water quality)	Wind direction and speed	
	Wave height	
	Tidal stage	
	Beaufort scale	
	State of floating debris on the surface	
	Location and identity of nearest hydrologic gauge	

13.0 Waste Management

13.1 The procedures described in this method generate minimal amounts of waste, if any, and no hazardous reagents or solvents are used. All waste including used foil, filters, labels, etc. can be disposed of in solid waste intended for landfill.

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APPENDIX A: PROTOCOL FIELD TESTING SUMMARY

Study Design

Experts identified variables unique to environmental water sampling that would potentially have strong influences on method performance and results: sample volume and sampling device. Experts agreed that sample volume selection should be driven by the predicted range of microplastic concentrations, the estimated levels of background contamination, and the desired level of statistical confidence. At the same time, practical limitations on sample volume due to environmental and sampling conditions (e.g., filter clogging) must be considered. Experts also recognized that no single sampling device will be applicable to all sampling scenarios and monitoring goals. Specifically, a variety of pumps with different mechanisms may be utilized for sampling. There was some concern amongst experts that some types of pump mechanisms may grind and fragment microplastic, leading to artificially high particle counts. There was also interest in comparing results across pump and net sampling.

To evaluate the robustness of the draft protocol and address the previously described uncertainties, the draft protocol was field-tested in a semi-controlled experiment at the International Institute of Sustainable Development – Experimental Lakes Area. The study methods and results are briefly summarized in this report but are described in detail elsewhere (Li Ludeña et al., *In Preparation*). Briefly, microplastic fragments ranging from ~35-1400 µm were added to an experimental lake in loading amounts intended to simulate microplastics entering the lake from stormwater runoff over a three-year period. Added plastics were brightly colored to be easily distinguishable from environmental microplastics and therefore rapid sample analysis. To compare how results varied by sampling equipment, 20 L samples of water were collected in duplicate using a peristaltic pump, a gear pump, and a submersible well pump. In addition, a surface water sample was collected using a manta trawl outfitted with a 53 µm mesh. The peristaltic pump was also tested using two different tube sizes (inner diameter 6.3 mm and 9.5 mm). The influence of target volume was tested by sampling five different volumes using the peristaltic and submersible pumps (i.e., 10, 20, 40, 80, and 160 L).

Results

Sampling Device

Overall, there were no significant differences amongst sampling devices in determining the concentration of microplastics >53 µm added to the lake. There were also no differences in the composition of microplastics captured for polymer type or size distribution. Tube diameter also

did not influence the number or composition of microplastics captured. Other studies have also found that different pump types produced similar results for collecting microplastic samples (Ugwu et al., 2024). Previous comparisons between pump and manta trawl sampling often demonstrate differences in the total number and composition of microplastics captured, particularly when the minimum mesh size is not controlled (Setälä et al. 2016; Hung et al. 2020; Schönlau et al. 2020; De-la-Torre et al. 2022; Du et al. 2022; Shi et al. 2023; Ueda et al. 2025; Yu et al. 2025). The similarity amongst samples observed in this field test is likely attributed to controlling the minimum mesh size (i.e., 53 µm). Additionally, manta trawl and pump sampling were conducted within similar portions of the water column at the surface and near-surface, respectively. Together, these results suggest that when the minimum mesh size and sampling depth are held relatively constant, the pump and net sampling devices tested here capture a comparable subpopulation of microplastics and produce similar results.

Variability amongst replicate samples was also comparable amongst sampling devices except for the gear pump where the standard error for amongst replicates for the total microplastics captured was more than six times greater than the other instruments. This is likely due to the sample directly interacting with the rotating gears of the pump, causing contamination or fragmenting microplastic particles (Li Ludeña et al., *In Preparation*). The sample also encountered pump gears in the submersible pump, but increases in variability were not observed in the results of the field test. The potential for contamination and fragmentation should be considered when selecting a pump for microplastics sampling, but sample contact with pump mechanisms should not be prohibitive.

Sample Volume

Volume is the most important parameter when sampling water for microplastics analysis. Sample volume determines the likelihood of capturing a given number microplastic particles and thus directly influences the accuracy and representativeness of the results. Larger volumes are more likely to detect microplastics and provide an accurate result. However, methodological, logistical, and cost constraints often limit sample volumes. Even if large sample volumes can be collected, higher concentrations of interferences may cause sample processing and analytical challenges. Recommendations for the minimum collection volume have been made based on matrix, target subpopulation of microplastics, and monitoring goals (as summarized by Cross et al., 2025). More recently, a Representative Sample Volume Predictor (RSVP) tool has also been developed to allow researchers to estimate a target minimum collection volume based on the target number of microplastics to be captured with a specified level of confidence (Cross et al., 2025).

Sample volume did impact the observed microplastic concentration as has been observed previously (Tamming et al., 2019; Karlsson et al., 2020). The lowest sampled volume (i.e., 10 L)

had the highest estimated microplastic concentrations whereas volumes 20 L and greater produced consistent estimated concentrations. These observations align with previous findings that insufficient volumes produce overestimations of microplastic concentrations (Cross et al., 2025). This result was also verified by the Representative Sample Volume Prediction (RSVP) tool, which also estimated 20 L to be the minimum representative volume when the concentration of microplastics in the field test was estimated to be ~6 particles/L and the minimum target number of particles to capture was set at 100. However, this is not to say that this volume is applicable for all monitoring scenarios. The optimum representative sample volume is a function of the targeted microplastic subpopulation and its concentration, and observed microplastic concentrations in environmental waters span several orders of magnitude (Cross et al., 2025). Thus, it is very helpful for researchers to have a sense of 1) the range of concentrations that may be detected in the field and 2) the level of background contamination likely to occur during sampling and laboratory analysis to ensure the selected volume captures a sufficient number of microplastics to achieve a detectable level of microplastics (i.e., exceed the minimum detectable amount, see limits of detection section for more detail) at the desired confidence level. This may be informed by investigating microplastic occurrence data in comparable habitats or conducting a small-scale pilot study prior to initiating a large-scale campaign. Researchers may also take advantage of resources such as the previously mentioned RSVP tool (Cross et al., 2025).

Feasibility

Field tests also allowed the protocol to be evaluated for feasibility and highlight important logistical, time, and cost considerations for collecting environmental water samples for microplastics analysis. The sampling devices performed similarly in the field test under similar conditions. However, they each provide advantages and disadvantages depending on monitoring goals and sampling conditions. For example, the gear pump was the least expensive but produced the greatest amount of variability amongst replicate samples. Many pumps also have a maximum depth at which they can be operated. It should also be noted that all pumps required an external power supply, which may be a challenge when sampling in remote locations. See Li Ludeña et al., (*In Preparation*) for in-depth comparison across all pump types tested in this study.

Limitations

Field testing demonstrated similar performance of all sampling devices tested and their permutations (i.e., inner tube diameter) and highlighted the importance of collecting a sufficient volume of water to avoid overestimating microplastic concentrations. However, the protocol was tested under a limited series of conditions and sampling parameters in a semi-controlled setting. First, sampling during the field testing was conducted in relatively calm

waters. Sampling in rough water or during inclement weather may produce significant challenges, particularly for the mitigation of background contamination. Secondly, microplastics used in the tests had a limited diversity relative to environmental microplastics. Particles were brightly colored and were comprised of three polymer types (i.e., polyethylene, polystyrene, and polyethylene terephthalate), a single morphology (i.e., fragments) and had a wide, but limited, size range (i.e., ~35-1400 µm). It should also be noted that in this pilot test a small number of replicates were collected for each permutation (n = 2-3), resulting in decreased statistical power. This sample collection protocol is intended to have the flexibility to adapt to a range of monitoring goals and logistical constraints. However, pilot testing is recommended if it is to be applied in particularly unique or extreme sampling scenarios.

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