Supplemental Materials C

Standard Operating Procedures for Sample Collection for Microplastics Analysis

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Standard Operating Procedures for the Collection of Ambient Water Samples for Microplastics Analysis

Prepared by

Southern California Coastal Water Research Project Authority

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1.0 Scope and Application

1.1 This method is for the collection of ambient water 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.

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 ambient water have been tested and described in other related work (see 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.

2.0 Summary of Method

This method describes the environmental sampling of microplastics from ambient water in lakes, bays, estuaries, and oceans (this protocol is not to be used in rivers, as there is a separate protocol for that). This method captures microplastics and other solids 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 (pumps and surface trawls) were selected because they are commonly used ambient 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, lwater 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. See figure below for more guidance.

Method	Decisions				
Pump	**Type of Pump**	**Tubing**	**Size Fractionation**	**Sample Volume**	**Depth of Sampling**
To be prioritized if only one method is used	e.g., peristaltic, gear, submersible. Different pumps may be more feasible depending on sampling depth, sampling location. Test your pump for contamination and recovery.	Use silicon or Tygon tubing. The inner diameter should be 6mm – 15mm (and at least 3x the size of the largest particle of interest).	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.	The volume should be enough to ensure that a) the amount of MPs in the sample are at least above the MDA.	Take a depth-integrated sample from just below the surface to 1m depth. Sampling can also be taken from other depths to meet monitoring objectives, e.g., discrete depths to sample a depth profile.
Surface Trawl	**Type of Trawl**	**Size of Mesh**	**Sampling Time**		
To be used in addition if there is interest in sampling the air-water interface	Use a manta trawl with a 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.	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 one could choose a 100 µm mesh.	The sampling time will determine the sample volume. The volume should be enough to ensure that a) the amount of MPs in the sample are at least 3x above blank levels and b) that the coefficient of variability between duplicate samples is < 30%.		

3.0 Definitions

Field blank (FB) – An aliquot of MAG water (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 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 (i.e., each monitoring day or every 10 field collected samples, whichever is more frequent). Collection of FB should happen independently for each sampling method (i.e., pump and surface trawl if both are used). 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. 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). 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.

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 Wang, 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 (e.g., pump, trawl) and sampling device.

Trip Blank – A sample of MAG water of at least 1 L, taken from the laboratory to the sampling site and returned unopened (i.e., without having been exposed to sampling 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 sample collection period. A Trip Blank must be evaluated prior to the sampling event if different sampling containers from those listed in Section 6 are used.

[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.

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 must use as much plastic-free equipment and consumables as possible, except where allowed as noted in Sections 4.2.2.3 to 4.2.2.8.

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).

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 4.2.1.3). 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 apparatuses shall be evaluated on a monthly basis for potential to shed microplastics by 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. When possible, it is recommended to collect samples of gear and equipment to compare to particles found in field samples.

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, orange) to allow clear identification within samples as contamination.

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 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 field expedition, wash all glassware, plasticware, and other 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 During each field expedition, rinse all glassware, plasticware, and tools with MAG water three times between each sample collected.

4.2.5.3 Cover all equipment (e.g., with clean aluminum foil) when not in use in the field or when stored.

5.0 Safety

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

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

5.3 Nitrile gloves are required for this method (Section 9 – Procedure) to minimize contamination from analysts.

6.0 Equipment and Supplies

6.1 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 labelling tape	Fisher Catalog no. 15901A
Writing utensil	
Data sheets	
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)	
Bucket with volume markings – check precision and consider error in any error budget.	
Sampling jars (for stacked sieves) or glass petri dishes (for in-line filtration)	
Stainless steel mesh sieves (for stacked sieves)	50 µm, 150 µm, 300 µm (recommended)
Tubing	Silicon or Tygon® (6 – 15mm ID)
Weights for the bottom of the tubing (metal)	
In-line filter holders (for in-line filtration)	
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	Can be purchased from any drug store
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
Writing utensil	
Data sheets	
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Surface trawl w/cod end	
Flow meter	
Sampling jars (glass recommended with heavy duty foil barrier used between the lid and jar)	
MAG water	
Bubble wrap	-
Cooler or heavy-duty storage container	-
99% Isopropyl Alcohol	Can be purchased from any drug store

7.0 Reagents and Standards

7.1 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 4.2.4.1) and covered (Section 4.2.4.3) until use.

7.2 The Field Blank(s) and Trip Blank(s) should be created by the laboratory using MAG water and clean (Section 4.2.4.1) glass or metal containers.

8.0 Quality Control

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.

8.1 Field Blank (FB) – A FB must be included with each sampling event and analyzed to assess contamination during shipping, sampling, and storage. 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.

8.2 Trip Blank – One Trip Blank must be used for each sampling event. They do not need to be analyzed unless the FB shows evidence of contamination. The Trip Blank may be analyzed to determine if contamination occurred during shipping or travel.

8.3 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 11.2). Cover the opening of the jar with pre-kilned heavy-duty foil and store according to Section 11.1. The sample may then be shipped (Section 11.2) 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 immediately prior to each sampling event or monthly, whichever occurs less frequently (Section 4.2.1.8).

8.4 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.

8.5 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.

9.0 Procedure

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

9.1 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.

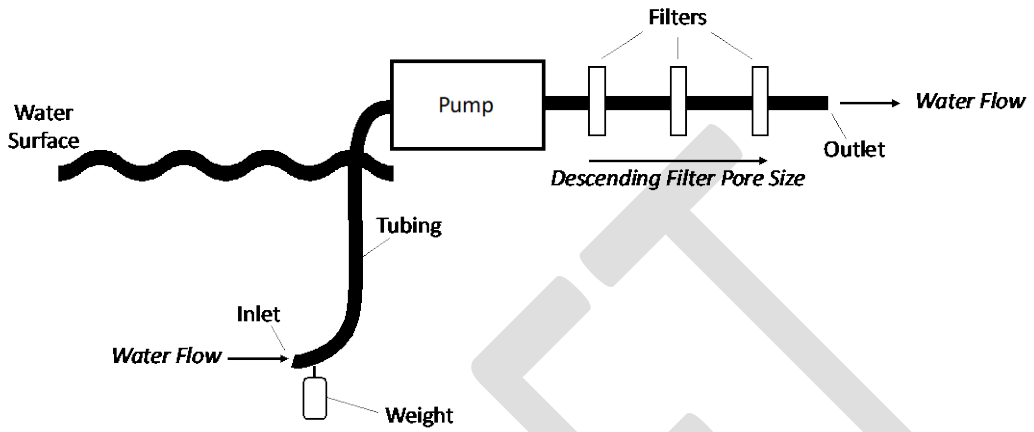
Samples can be taken at the depth of choice based on the monitoring question of interest. For example, a sub-surface sample can be taken by holding the tube 10 cm below the surface. Alternatively, a depth-integrated sample can be taken by moving the tube or pump up and down slowly and steadily from recorded 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 a) 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 program to assess the volume needed to meet these criteria. The supplementary MDA and Sampling Unit Calculator may be used to calculate MDAs and estimate sample volumes needed to exceed the MDA.

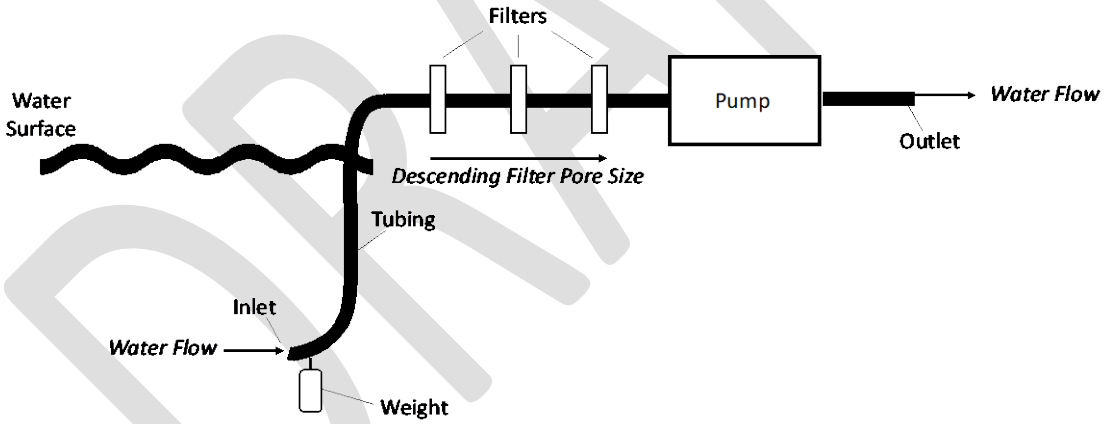
Microplastics may be collected either by pumping water through a sieve stack or a series of in-line mesh filters (see figure below). Using a sieve stack may help reduce clogging, whereas using in-line filtration may help reduce sample contamination. When filtering using a sieve stack, it is recommended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

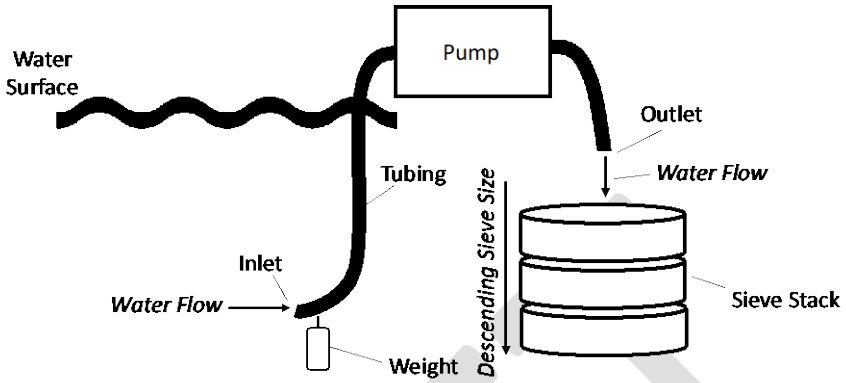
Pump Upstream of Filters



Pump Downstream of Filters



Pump Sampling w/sieve stack



9.1.1 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.

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

9.1.3 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).

9.1.4 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).

9.1.5 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.

9.1.6 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.

9.1.7 If using a sieve stack, add 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 RO water with alcohol as well (choose an alcohol that is compatible with your filter material).

9.2 Sampling via a surface trawl

Note: 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.

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., see Michida et al., 2023).

9.2.1 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.

9.2.2 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.

9.2.3 Attach the flow meter to the net 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.

9.2.4 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.

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

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

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

9.2.8 Add 10% by volume 99% isopropyl alcohol to the sample.

10.0 Sample Preservation and Storage

10.1 Samples can be stored at room temperature, but an appropriate alcohol should be added (to a volume of 10% of the sample) to liquid samples to prevent growth of biotic material. 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.

10.2 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. Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts 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.

10.3 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.

10.4 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.

11.0 Field Data Reporting Requirements

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

Sampling data and location	Sampling date and 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	Wind direction and speed	
	Wave height	
	Beaufort scale	

conditions, water quality)	State of floating debris on the sea surface

12.0 Waste Management

12.1 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.

13.0 References

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Standard Operating Procedures for the Collection of Riverine Water Samples for Microplastics Analysis

23 July 2024

1.0 Scope and Application

1.1 This method is for the collection of water samples from rivers for the determination of the concentration and composition of microplastics, as defined by the California State Water Resources Control Board (2020). The method is based on peer-reviewed literature and recommendations from an expert panel and work group convened and coordinated by the International Joint Commission (IJC).

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 California State Water Resources Control Board (State Water Resources Control Board 2022a, 2022b). Procedures for the extraction and measurement of microplastic particles in ambient water and sediments (relevant here) have been tested and described in other related work (see 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.

2.0 Summary of Method

This method is adapted from commonly used sampling techniques for microplastics in rivers. It describes the in-situ filtration of microplastics from river water and uses bulk water sampling via pumps. 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., location, volume, what type of pump to use, sampling depth, whether to include sediment traps, etc.). For example, the choice of pump is dependent on sampling and logistical conditions (e.g., depth, cost, power supply) and the type and size of tubing dependent on pump type and the largest size of particles that will be collected. See figure below to guide decisions. If relevant, a monitoring program could add an adapted manta trawl method informed by the ambient water protocol. If a manta trawl is used, safety should be considered because these methods are more difficult in flowing water. When choosing sampling sites, we recommend sites near stream gauges to provide reliable measures of flow to get estimates of microplastics loadings.

Method	Decisions				
Pump	Type of Pump	Tubing	Size Fractionation	Sample Volume	Depth of Sampling
To be prioritized if only one method is used	e.g., peristaltic, gear, submersible. Different pumps may be more feasible depending on sampling depth, sampling location. Test your pump for contamination and recovery.	Use silicon or Tygon tubing. The inner diameter should be 6 mm – 15 mm (and at least 3x the size of the largest particle of interest).	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.	The volume should be enough to ensure that the amount of MPs in the sample are at least above the minimum detectable amount (MDA).	Take a depth-integrated sample from just below the surface to just above the bed. Sampling can also be taken from other depths to meet monitoring objectives, e.g., discrete depths to sample a depth profile.

3.0 Definitions

Field blank (FB) – An aliquot of MAG water (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 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 monitoring day or every 10 field collected samples, whichever is more frequent. This should happen independently for both sampling methods (i.e., pump and sediment trap if both are used). 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 4 L. FBs must be analyzed alongside field collected samples from the same sampling event. As such, the FB may also serve as the Laboratory Reagent Blank (LRB). If this approach is used, it is recommended that a subset of LRBs are 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). FBs differ from Trip Blanks (see definition below) in that the FB evaluates contamination during both sample collection and sample transport, while the Trip Blank only accounts for contamination during transport (i.e., shipment and storage).

Microplastics – Solid[1] polymeric materials[2] to which 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 5000

[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

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 1 µm 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 equipment. 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 Wang, 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 (e.g., pump, trawl) and sampling device.

Trip Blank – A sample of MAG water of at least 1 L, taken from the laboratory to the sampling site and returned unopened (i.e., without having been exposed to sampling 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 sample collection period.

4.0 Interferences

4.1 Physical Interferences

4.1.1 Preventing samples from becoming contaminated during collection can become one of the greatest difficulties encountered in quantifying microplastics in environmental 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 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

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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.

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 must use as much plastic-free equipment and consumables as possible, except where allowed as noted in Sections 4.2.2.3 to 4.2.2.8.

4.2.2.1 Field crews and laboratory personnel must use equipment composed of glass (e.g., sampling jars) or metal (e.g., foil, sampling devices, scoops) throughout the process, 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).

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 must 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 If plastic materials are used, inspect their integrity. For example, potential contamination from sampling jars and lids should be tested prior to choosing the jars and lids to be used. 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 – Equipment and Supplies.

4.2.2.8 All plastic apparatuses shall be evaluated on a monthly basis for potential to shed microplastics by 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, as well as anything relevant in the sampling locations, for plastic debris or other potential sources of particle contamination. Remove

or replace materials as necessary. When possible, it is recommended to collect samples of gear and equipment to compare to particles found in field samples.

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, orange) to allow clear identification within samples as contamination.

4.2.4.2 If Personal Protective Equipment (PPE) must be worn for safety reasons, it is recommended that field crews document the type 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 field expedition, wash all glassware, plasticware, and other 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 During each field expedition, rinse all glassware, plasticware, and tools with MAG water three times between each sample collected.

4.2.5.3 Cover all equipment (e.g., with clean aluminum foil) when not in use in the field or when stored.

5.0 Safety

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

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

5.3 Nitrile gloves are required for this method (Section 9 – Procedure) to minimize contamination from handling.

6.0 Equipment and Supplies

6.1 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
Writing utensil	
Data sheets	
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Pump with power source (may be a battery that needs to be brought on board and charged)	
Bucket with volume markings – check precision and consider error in any error budget	
Sampling jars (for stacked sieves) or glass petri dishes (for in-line filtration)	
Stainless steel mesh sieves (for stacked sieves)	50 µm, 150 µm, 300 µm (recommended)
Tubing	Silicon or Tygon® (6 – 15 mm ID)
Weights for the bottom of the tubing (metal)	
In-line filter holders (for in-line filtration)	
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	Can be purchased from any drug store

7.0 Reagents and Standards

7.1 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 (Section 4.2.3) glass or metal vessel and covered (Section 4.2.5.3) until use. It can be temporarily kept in and dispensed from a Teflon, polypropylene, or LDPE squeeze bottle (Section 4.2.2.4).

7.2 The Field Blank(s) and Trip Blank(s) should be created by the laboratory using MAG water and clean (Section 4.2.3) glass or metal containers.

8.0 Quality Control

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.

8.1 Field Blank (FB) – One FB must be included with each sampling event and analyzed to assess contamination during sample collection, shipping and storage. 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.

8.2 Trip Blank – One Trip Blank must be used for each sampling event. They do not need to be analyzed unless the FB shows evidence of contamination. The Trip Blank may be analyzed to determine if contamination occurred during shipping or storage.

8.3 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 and 10.3) 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 immediately prior to each sampling event or monthly, whichever occurs less frequently (Section 4.2.2.8).

8.4 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.

9.0 Procedure

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

9.1 Pump Sampling

There are several types of pumps that can be used. For example, a peristaltic or gear pump can be used with a weighted tube that is deployed from a bridge, boat, or while wading within the river. 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 in deeper rivers. 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.

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 sampling while 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. We recommend a depth-integrated sample from just below the surface to just above the bed, being careful not to sample bottom sediment.

Samples can be taken at various locations across the river horizontally during each sampling. The samples can then be combined as a composite if not taken at once. If it is only possible to take from one location across the river, the thalweg should be prioritized.

The sample volume needs to be enough to ensure that a) 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 low-microplastic regions, volumes of >100 L may be needed. In high-microplastic regions, volumes may only need to be ~20 L. It is recommended to start with a pilot sampling program to assess the volume needed to match this criteria.

Microplastics may be collected either by pumping water through a sieve stack or through a series of in-line mesh filters (Figures 1 and 2). Using a sieve stack may help reduce clogging, whereas in-line filtration may help reduce sample contamination. If 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 (Figure 2), rather than downstream to avoid potential contamination from the pump.

Pump Sampling w/sieve stack

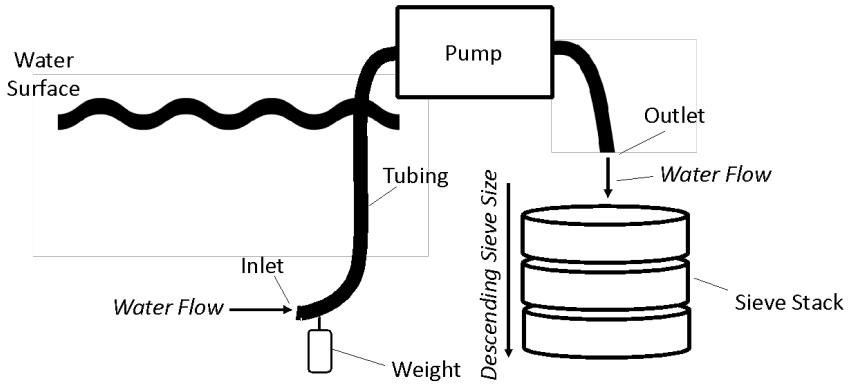

Figure 1. Schematic diagram of microplastic collection in riverine water via a sieve stack.

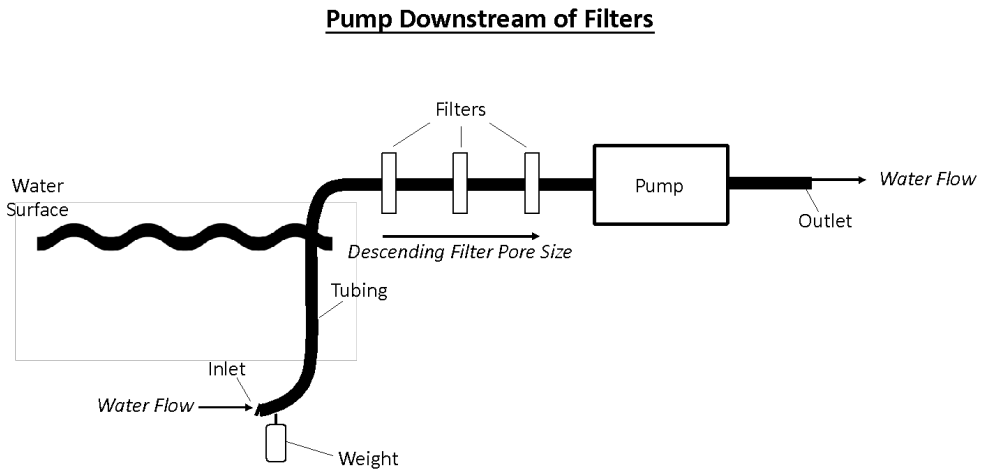

Figure 2. Schematic diagram of microplastic collection in riverine water via in-line filters (A) with the filters upstream of the pump, rather than having the filters downstream of the pump.

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

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

9.1.3 Prime 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).

9.1.4 If using a sieve stack, stack the clean sieves with the smallest size on bottom and largest on top. 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).

9.1.5 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. To keep track of volume of water sampled, place the sieve stack on top of a marked bucket or the output tube inside the marked bucket. After the sample is processed, record the volume of water in the bucket. Another method would be to calibrate the pump to determine the pumping rate (L/min). 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. Record the depth sampled, the time it takes to pump the sample, and the volume of water filtered.

9.1.6 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 forceps to remove each filter, placing them in labeled, clean glass petri dishes.

9.1.7 If using a sieve stack, add 99% isopropyl alcohol to a volume of 10% of the sample to each sample jar.

10.0 Sample Preservation and Storage

10.1 Samples can be stored at room temperature, but 99% isopropyl alcohol should be added (to a volume of 10% of the sample) to liquid samples to prevent growth of biotic material. 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 to prevent UV or photodegradation of plastics.

10.2 Glass containers with non-plastic lid liners (PTFE is acceptable), pre-cleaned as with other apparatuses (Section 4.2.3) in this method, must be used to collect and store samples to minimize microplastic contamination from the container when feasible. Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts 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.

10.3 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; they 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 from storage, and do not need to be analyzed unless evidence of contamination arises from analysis of FBs.

10.4 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 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.

11.0 Field Data Reporting Requirements

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

Sampling data and location	Sampling date and 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
Pumping parameters	*Sampling via a pump*	Pump speed or pump duration
		Filtered water volume
		Depth sampled
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	
	Flow rate/Discharge	
	Substrate Type (fine, sand, cobble, pebbles)	
	Time since last wet event (>10 mm of precipitation)	

12.0 Waste Management

12.1 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.

13.0 References

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Standard Operating Procedures for the Collection of Aquatic Biota Samples for Microplastics Analysis

Prepared by

Southern California Coastal Water Research Project Authority

27 June 2024

1.0 Scope and Application

1.1 This method is for the collection of biota for the determination of the concentration and composition of microplastics, as defined by the State of California 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.

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).

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 ensuring that all procedures are in accordance with best practices and institutional requirements and approvals for animal collection and sacrifice are met (e.g., approval or procedures by Institutional Animal Care and Use committee).

1.6 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 is adapted from commonly used sampling techniques for biota for other assessment types (e.g., chemical contaminants, fish surveys, etc.) with specific modifications for microplastics sampling included. This document provides guidance on the choice of taxa based on the scientific objectives of the study. This document also provides guidance on the number of organisms to be collected based on the acceptable levels of variation and statistical power, within the desired habitat types, and predicted level of microplastic contamination. Specific methods are provided for bivalves and fish, but general principles (e.g., background contamination mitigation procedures) may be applied to other classes of aquatic organisms if desired.

3.0 Definitions

Dissection / processing? blank (DB) – An aliquot of MAG (Microplastics Analysis Grade, see definition below) water that is placed in a sample container or a wetted filter placed in a pre-cleaned petri dish. If using a wetted filter, the pore size should be less than the lower particle size limit of the study. The purpose of the DB is to determine if method analytes or other interferences are introduced into the samples during dissection. This method assumes that internal tissues (e.g., digestive tract, muscle fillet) are the primary target for microplastics analysis. Therefore, it is assumed that there is little to no risk of particle contamination until organisms are dissected and tissues are exposed to the external

environment. The DB is treated as a sample in all respects, including exposure to dissection conditions, storage, preservation, and all analytical procedures. At least one DB must be used for each dissection event and analyzed alongside field collected samples. DBs differ from Trip Blanks in that the DB evaluates contamination during dissection, while the Trip Blank only accounts for contamination during shipment. Given that the DB is analyzed alongside field collected samples, the DB 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).

Microplastics – Solid[1] polymeric materials[2] to which 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 1 µm or smaller. Filters must be of an appropriate material that does not shed particles (Section 4.2 and 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).

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[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.

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 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 sample collection period. A Trip Blank must be evaluated prior to the sampling event if different sampling containers from those listed in Section 8 are used.

4.0 Interferences

4.1 Physical Interferences

4.1.1 Preventing samples from becoming contaminated is one of the greatest difficulties in quantifying microplastics. It is not possible to confidently eliminate all contamination from samples, however quantifying the contamination is possible. It is important that extreme care be taken to minimize contamination when collecting biota samples for microplastics, particularly during dissection. 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 during sample collection include, but are not limited to: fibers from clothing and textiles (including lab coats, synthetic ropes and lines on sampling vessels and equipment, apparel worn by field personnel, carpets, and furniture), 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 container 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 must use as much plastic-free equipment as possible, except where allowed as noted in Sections 4.2.2.3 to 4.2.2.6.

4.2.2.1 Field crews and laboratory personnel must use equipment throughout the process composed of glass or metal (e.g., foil, sampling devices, scoops), except as noted in Sections 4.2.2.3 to 4.2.2.6.

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).

4.2.2.3 Use of hard plastic tubing (e.g., Tygon® or clear PVC tubing) to dispense water used to make MAG water is acceptable.

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., DBs 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 Tissues may be directly placed into polypropylene jars if isolated upon collection (e.g., dissection in the field). Polypropylene is used because potassium hydroxide, which is used to digest tissues, will sometimes etch glass.

4.2.2.7 If plastic materials are used, inspect their integrity. Examples of plastics commonly used in microplastics analysis that are acceptable as they do not shed polymer particles are listed in Sections 4.2.2.3 and 4.2.2.6.

4.2.2.8 All plastic apparatus shall be evaluated for potential to shed microplastics by the procedures noted in Section 11 – Quality Control. Conduct this verification monthly. If sampling occurs less frequently than once a month, conduct this verification immediately prior to each sampling event.

4.2.3 Clean all equipment thoroughly before use.

4.2.3.1 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.

4.2.3.2 During each sampling event, rinse all glassware and tools with MAG water between each organism collected and dissected.

4.2.3.3 Cover all equipment with clean aluminum foil when not in use in the field or when stored.

4.2.4 It is highly recommended that collected organisms are dissected in a clean laboratory environment under a hood or in a clean cabinet with HEPA filtration. If organisms must be dissected in the field, precautions to mitigate and analyze background contamination must be taken.

4.2.4.1 A DB must be used to characterize background contamination during dissections both in the field and the laboratory. It should be placed as close as reasonably possible to the working area.

4.2.4.2 Ensure a clean working environment before and during biota collection. Inspect sampling gear and equipment onboard boats or areas near the sampling location for plastic debris or other potential sources of particle contamination in the working area where dissections will take place. Remove or replace materials as necessary. If removal is not possible, it is recommended that field crews document potential sources of contamination by taking photographs, taking detailed notes, and collecting samples to compare to particles found in samples.

4.2.4.3 Minimize the use of synthetic textiles in the field. It is recommended that field crews do not wear synthetic clothing when collecting samples. If possible, wear cotton laboratory coats (or similar garment 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 of the particles within samples as contamination. Exceptions may be made for extreme weather conditions (e.g., wet; 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.

4.2.4.4 If 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.

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 are required for this method to minimize contamination from analysts (see Section 4.2.2.5).

5.5 Safety glasses must be worn while chiseling bivalves from hard substrates (see Section 8.3.2.2).

6.0 Taxa Selection

The selection of taxa targeted for sampling should be carefully considered early in the project scoping and planning phase and should be driven by the specific scientific objectives and questions of the monitoring program. Some examples of potential questions and recommended taxa for microplastic monitoring may include, but are not limited to:

- What is the exposure of pelagic organisms to microplastics?
    - Recommended taxa: forage fish (e.g., anchovies, topsmelt)
- What is the exposure of sediment-dwelling organisms to microplastics?
    - Recommended taxa: clams, demersal fish (e.g., flatfish)
- What is human exposure to microplastics through subsistence fishing?
    - Recommended taxa: oysters, mussels, clams, sport fish

If internalized microplastics are to be targeted for analysis, it is important to consider the ingestibility of different size classes of microplastic particles. Specifically, organisms are only capable of ingesting particles smaller than the maximum gape size in at least one dimension (Koelmans et al., 2020), and bivalves are known to reject particles of specific morphologies and sizes (Ward et al., 2019). The likelihood of ingestion will also depend on the environmental conditions and ecology of the target organisms (e.g., diet, gape size, habitat use, feeding behavior). These variables must be carefully considered during the design phase to ensure that the sampling strategy is appropriate to meet desired objectives.

Data on the general status/condition of the biota sampled should be collected to better contextualize microplastics results. This may include, but is not limited to, body mass, body length, condition factor, sex, estimated age, and occurrence of abnormalities.

7.0 Sample Size and Tissue Mass Recommendations

Target sample sizes must be determined during monitoring planning stages, well in advance of sample collection. Sample sizes required to achieve acceptable levels of variation and statistical power will depend on a variety of factors including the target species, microplastic ingestion rates, microplastic contamination levels within target sites, the lower particle size limit of detection, and the goals of the specific monitoring campaign.

Previous guidelines for fish and bivalves have recommended at least 50 individual organisms for each experimental permutation (e.g., species, feeding type, habitat, etc.) (Hermsen et al., 2018, ICES 2015, Galgani et al., 2013) though in some cases, sample sizes as small as ten individuals per site has provided enough statistical power to detect differences amongst sampling sites (Miller et al., 2021).

The amount of biomass per individual will vary depending on the target species and whether individual organisms versus composite samples are analyzed. However, it is recommended that each sample is comprised of at least 5 g of tissue (wet weight) for fish and bivalves. For taxa with small body sizes (e.g., aquatic invertebrates), 2 g of tissue (wet weight) is recommended, if possible (Torres et al., 2023).

If more granular data are desired, individual organisms should be analyzed as opposed to composite samples, which would provide more integrative data (Miller et al., 2021). Prior to sampling, a brief literature review should be conducted to determine if there is any data available on microplastic contamination for the target species, sampling location, or habitat type. If so, it is recommended that a power analysis is conducted to estimate sample sizes required to achieve monitoring objectives.

Determination of sample sizes and target biomass may be facilitated by using the supplementary MDA and Sampling Unit Calculator. This tool may be used to calculate MDAs and estimate the amount of tissue required for analysis to ensure detectable amounts of microplastics.

8.0 Bivalve Collection: Equipment, Supplies, and Procedures

8.1 Equipment and supplies for the collection of bivalves 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	47 mm diameter, Sterlitech Polycarbonate Track Etch (PCTE) membrane filters, Catalog no. PCT1047100
Mussel and Oyster Sample Collection	
Global Positioning System (GPS)	-
Nitrile gloves	Kimtech™ Purple Nitrile™ Exam Gloves (Product code #55083)

Pre-ashed heavy-duty aluminum foil	-
Laboratory labelling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Measuring tape or ruler	-
Rubber mallet	-
Chisel	-
Safety glasses	-
Ethanol	-
Wet ice	-
Cooler or heavy-duty storage container	-
Clam Sample Collection	
GPS	-
Nitrile gloves	Kimtech™ Purple Nitrile™ Exam Gloves (Product code #55083)
Metal spade or rake	-
Dip net	-
Pre-ashed heavy-duty aluminum foil	-
Laboratory labelling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Measuring tape or ruler	-
Ethanol	-
Wet ice	-
Cooler or heavy-duty storage container	-
Bivalve Cleaning and Shucking	
Nitrile gloves	Kimtech™ Purple Nitrile™ Exam Gloves (Product code #55083)
Natural fiber scrub brush	Amazon – "Naturolic All-Natural Wooden Scrub Brush"
Buckets	-
Glass petri dish	VWR Catalog no. 25354-069
20 µm pore-size filters (or pore size smaller than the lower particle size limit)	Suggestion: Sterlitech Catalog no. 1270175
Pre-ashed heavy-duty aluminum foil	-
Heavy-duty gardening gloves	Grainger (Item 56FK94)
Shucking knife	-
Calipers	VWR Catalog no. 36934-152
Metal Scissors	-
Balance	-
Polypropylene sample jars or glass sample jars (Note: If tissues are digested using potassium hydroxide, glass may etch)	Jar, Straight Sided, Polypropylene, Dynalon; VWR, (Catalog no. 30617-164) OR Ball 16oz 12pk Glass Wide Mouth Mason Jar with Lid and Band, Target OR 16oz Glass Wide Mouth Sampling Jars, Environmental Sampling Supply

	(Part #0500-0015-QC)

8.2 The following procedures assume that all equipment and supplies have been cleaned according to previously described requirements (see Section 4.2.3).

8.3 Oyster and/or mussel collection. Oysters and mussels are collectively referred to as bivalves in this section.

8.3.1 Bivalves are to be collected at low tide when shells are closed to facilitate access to bivalve beds and reduce potential background contamination.

8.3.1.1 Locate the sampling site using GPS.

8.3.1.2 Locate bivalves within 200 meters of the target latitude. Bivalves may be embedded in the sediment or attached to hardscapes.

8.3.2 Collecting bivalves for microplastics analysis.

8.3.2.1 Some bivalves may be found in the sediment. Sometimes they are visible, other times they can be found by dragging a hand or chisel through the mud. These can be picked up with your hand.

8.3.2.2 If bivalves are attached to hardscapes, rest the sharp end of the chisel in between the oyster and substrate at about a 45-degree angle (Figure 1). Hit the handle of the chisel with moderate force with rubber mallet to wedge it in between the bivalve and substrate. If this does not work, hit it again with more force or try another angle.

Note: Rocks can have irregular surfaces that make it hard to place the chisel between the rock and oyster so reposition frequently. Be wary - this does increase the chances of piercing the shell.

Figure 1. Removal of bivalve from hardscape using mallet and chisel.

8.3.2.3 Upon collection, rinse each bivalve in ambient water to remove excess debris and wrap each individual bivalve in a piece of pre-ashed heavy-duty aluminum foil and place in a cooler on ice in the shade. All samples should be stored on ice or at 4°C until shucked.

8.4 Collecting clams for microplastics analysis.

8.4.1 Locate the sampling site using GPS.

8.4.2 Locate clams within 200 meters of the target latitude.

8.4.3 Collect clams. Clams may be detected by looking for small bubbling holes or dimples in the sediment. In shallow water, a metal spade or small rake may be used to skim the surface of the sediment. In deeper water, a dip net may be used. Clams may be buried up to 6 inches in sediment.

8.5 Cleaning and shucking bivalves (i.e., oysters, mussels, clams).

8.5.1 It is recommended that bivalves be cleaned and shucked within 48 hours of collection and never frozen. Freezing and thawing samples will make bivalves difficult to shuck and may lead to increased microplastic particle loss. If absolutely necessary, samples should be subjected to no more than one freeze-thaw cycle (Section 12.2). Alternatively, tissues may be preserved in 70-100% ethanol filtered through a pore-size of 1 µm or smaller.

8.5.2 It is highly recommended that bivalves are cleaned and shucked in a clean laboratory environment and not in the field unless absolutely necessary. It should be noted that cleaning and

shucking in the field is likely to result in greater levels of background contamination. See Section 4.2.4 for requirements and recommendations on dissecting organisms in the field.

8.5.3 If bivalves are frozen, thaw samples at 4°C for 24-48 hours, depending on the size of the organism. If organisms are not already in a sample jar (e.g., wrapped in foil), it is recommended that bivalves are placed in a clean, covered container (see Section 4.2.3) lined with pre-ashed heavy-duty foil in case of leakage. If leakage does occur, any liquid should be collected and processed with tissues to prevent particle loss.

8.5.4 Fill up a small bucket with tap water. Use the natural fiber scrub brush to clean mud, algae, and debris off the exterior of the bivalve, paying special attention to where the two shells meet. Periodically dip the bivalve into the bucket and use the spray hose to wash off debris. Bivalves are clean when the water runs clear.

8.5.4.1 Inspect bivalves during cleaning. Any bivalve with broken, pierced, or open shells must be discarded.

8.5.5 Set up the balance and a DB. Lay down a large piece of pre-ashed heavy-duty aluminum foil.

8.5.5.1 Set up equipment in a fume hood or clean cabinet if in the laboratory.

8.5.6 Rinse the outside of the bivalve with MAG water three times. One at a time, place the bivalve on the foil. Use the calipers to measure the shell length from the hinge to the top of the shell at the longest point. Record the length.

8.5.7 Shuck the bivalve.

8.5.7.1 Hold the shell firmly on the table with hand in a heavy-duty gardening glove. Rinse the shucking knife three times with MAG water. Use the shucking knife to pierce anywhere where the two shells meet. Pierce at the hinge. Pierce along the un-frilled side section of the shell (Figure 2).

Note: Be aware that shells may become brittle and break. Avoid touching the inside of the shell with anything except a shucking knife rinsed with MAG water.

8.5.7.2 Once pierced, shove the blade of the shucking knife ~50-70% into the bivalve. Use this to pry open the bivalve by twisting the knife until the shell opens. Slide the shucking knife until you reach the adductor muscle and cut through it. Open the shell.

8.5.8 Weigh a sample jar on the balance and record the mass. Tare the balance and use the shucking knife to detach the bivalve viscera from the shell. Use the knife to slide the viscera into the sample jar. Record the mass and close the jar.

8.5.9 Repeat steps 8.5.6 through 8.5.8 for the remaining bivalves to be sampled. If bivalves are to be pooled, viscera may be deposited into same sample jar, taring the balance each time, or subtracting the mass of the jar and other viscera post-hoc.

8.5.10 Close or cover the DB.

8.5.11 Store samples including DB at -20°C or in 100% pre-filtered ethanol at 4°C until processing and analysis. Note that samples should not undergo more than one freeze-thaw cycle (Section 12.2).

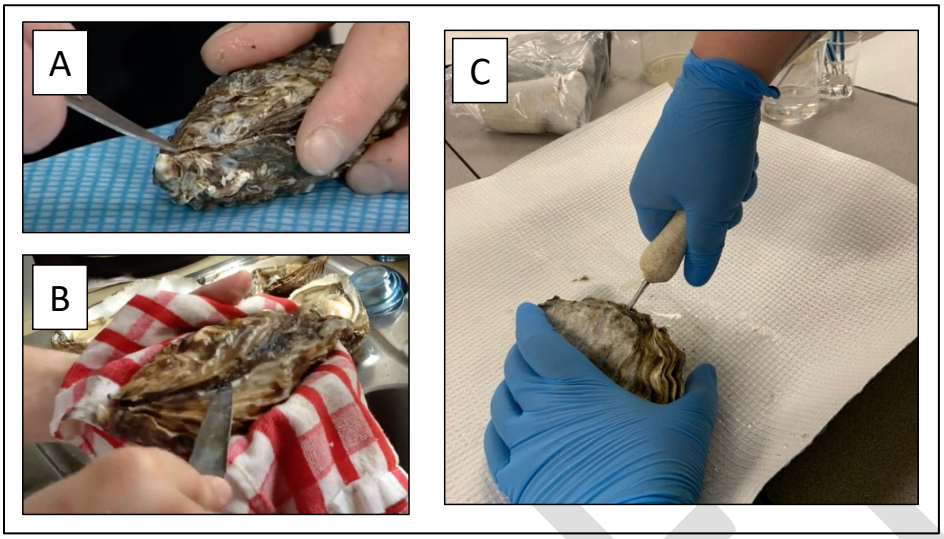

Figure 2. (A) Example of piercing from hinge. (B) Example of piercing from un-frilled side. (C) Example of how deep the shucking knife should be inserted into the bivalve for shucking.

9.0 Fish Collection: Equipment, Supplies, and Procedures

9.1 Equipment and supplies for the collection of fish 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**
Fish Sample Collection	
Nitrile gloves	Kimtech™ Purple Nitrile™ Exam Gloves (Product code #55083)
Pre-ashed heavy-duty aluminum foil	-
Laboratory labelling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Collection device of choice (e.g., seine net, hook and line, trawl net, etc.)	-
Wet ice	-
Cooler or heavy-duty storage container	-
Whole Fish Collection	
Calipers	VWR Catalog no. 36934-152
Kim Wipes	-
Balance	-
Polypropylene sample jars	VWR Catalog no. 30617-164
Dissection	
Pre-ashed heavy-duty aluminum foil	-
Calipers	VWR Catalog no. 36934-152
Kim Wipes	-

Balance	-
Dissection tools (e.g., scalpel, scissors, forceps)	-
Polypropylene sample jars or glass sample jars (Note: If tissues are digested using potassium hydroxide, glass may etch at higher concentrations or longer incubation times)	Jar, Straight Sided, Polypropylene, Dynalon; VWR, (Catalog no. 30617-164) OR Ball 16oz 12pk Glass Wide Mouth Mason Jar with Lid and Band, Target OR 16oz Glass Wide Mouth Sampling Jars, Environmental Sampling Supply (Part #0500-0015-QC)

9.2 The following procedures assume that all equipment and supplies have been cleaned according to previously described requirements (see Section 4.2.3).

9.3 The following procedures present options for analyzing whole fish as well as internalized microplastics (i.e., microplastics in the digestive tract, muscle, liver, etc.). The analysis of whole fish (Section 9.4.2) is only recommended for fish < 5g or species too small to reliably isolate tissues via dissection. If fish are > 5g, dissection and isolation of tissues targeted for microplastics analysis is recommended (Section 9.4.3) (Figure 3).

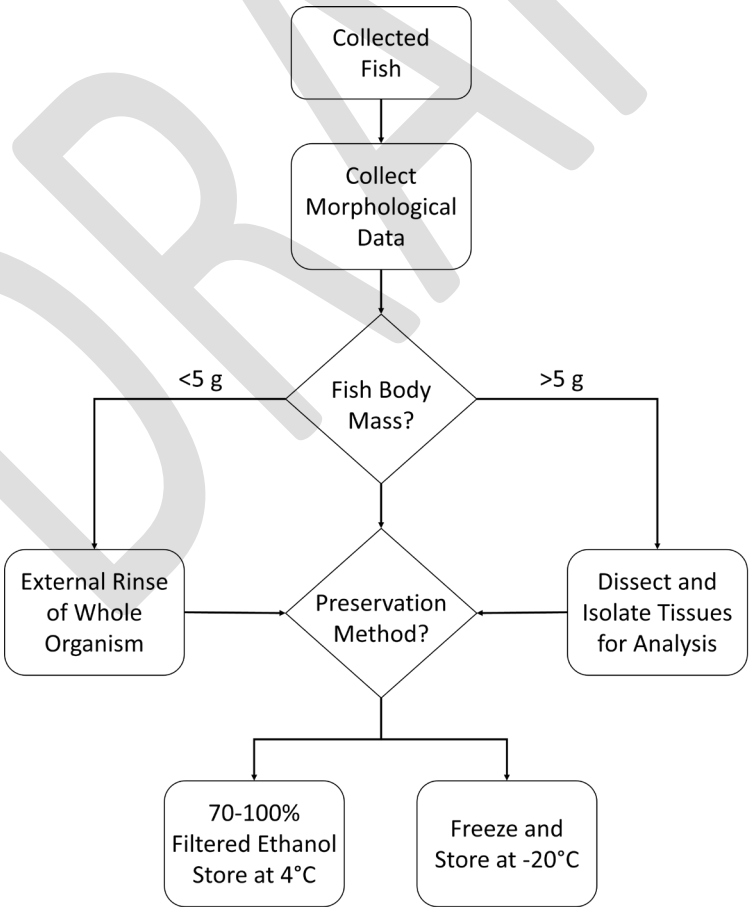

Figure 3. Decision tree for sampling and preserving fish on the basis of body size.

9.4 Fish collection.

9.4.1 Fish may be captured and sacrificed via the method appropriate for the targeted species. This may include, but is not limited to, electrofishing, trawling, seine netting, and angling (i.e., hook and line).

9.4.1.1 All devices and equipment that come into direct contact with fish during capture must be evaluated for their potential to shed microplastic particles as described in Section 11, Quality Control.

9.4.1.1.2 If netting, ropes or line is used, it is recommended that a sample be collected for the identification of material type and potential comparison to particles detected in blank samples.

9.4.2 Collection and preservation of whole fish in the field for microplastics analysis.

9.4.2.1 Immediately following capture and sacrifice of fish, remove the lid from the DB and place it as close to the collection site as possible. Make sure that the DB is on a stable surface and not in danger of being knocked over.

9.4.2.2 Place a clean, pre-ashed piece of heavy-duty aluminum foil on the balance. Tare the balance. Dab the outside of the fish with a clean Kim wipe to remove excess moisture and place the fish on the foil. Record the mass.

9.4.2.3 Use the calipers, ruler, or measuring board to measure total length (i.e., tip of the tail to snout) or standard length (i.e., fork to snout), whichever is desired. Record the length.

9.4.2.4 Record any other data associated with the condition or morphology of the fish (e.g., sex, maturity stage, presence of externally visible diseases, etc.)

9.4.2.5 Rinse the outside of the fish three times with MAG water. Open the lid of the sample jar. Place the fish in the sample jar and close the lid.

9.4.2.5.1 If fish are to be pooled as composite sample, repeat steps 9.4.2.2 through 9.4.2.5 until all fish are in the sample jar.

9.4.2.6 Close the DB.

9.4.2.7 Whole fish may also be kept on wet ice for up to 48 hours until they are stored at -20°C. Store samples at -20°C. Alternatively, samples may be preserved in 70-100% ethanol filtered through a pore-size of 1 µm or smaller and stored at 4°C until processing and analysis. Note that samples should not undergo more than one freeze-thaw cycle (Section 12.2).

9.4.3 Collection of internal organs and other tissues of fish for microplastics analysis.

9.4.3.1 It is recommended that fish are dissected within 48 hours of collection and not frozen prior to dissection if this is possible. Freezing and thawing samples will make fish

difficult to dissect and may lead to increased microplastic particle loss. If absolutely necessary, samples should be subjected to no more than one freeze-thaw cycle (Section 12.2). Alternatively, tissues may be preserved in ethanol filtered through a pore-size of 1 µm or smaller and stored at 4°C until processing and analysis.

9.4.3.2 It is highly recommended that fish are dissected in a clean laboratory environment and not in the field unless absolutely necessary. It should be noted that dissecting in the field is likely to result in greater levels of background contamination. See Section 4.2.4 for requirements and recommendations on dissecting organisms in the field.

9.4.3.3 Isolation of fish tissues via dissection for microplastics analysis.

9.4.3.3.1 Immediately following capture and sacrifice of fish, wrap each fish in pre-ashed heavy-duty aluminum foil and place on ice until return to the laboratory where they may be stored at 4°C for up to 48 hours. Samples should be dissected within 48 hours of collection unless they are to be frozen before dissection. If the latter, whole fish should be frozen as soon as possible.

9.4.3.3.2 If fish are frozen, thaw samples at 4°C for 24-48 hours, depending on the size of the fish. If organisms are not already in a sample jar (e.g., wrapped in foil), it is recommended that fish are placed in a clean, covered container (see Section 4.2.3) lined with pre-ashed heavy-duty foil in case of leakage. If leakage does occur, any liquid should be collected and processed with tissues to prevent particle loss.

9.4.3.3.3 Inside a clean cabinet or fume hood, ideally with HEPA filtration, set up the balance and a DB. Lay down a large piece of pre-ashed heavy-duty aluminum foil.

9.4.3.3.4 One at a time, remove the foil from the outside of the fish and rinse the outside of the fish with MAG water three times. Place the fish on a clean, pre-ashed piece of heavy-duty foil in a clean cabinet or under a fume hood.

9.4.3.3.5 Place a clean, pre-ashed piece of heavy-duty aluminum foil on the balance. Tare the balance. Dab the outside of the fish with a clean Kim wipe to remove excess moisture and place the fish on the foil. Record the mass.

9.4.3.3.6 Use the calipers, ruler, or measuring board to measure total length (i.e., tip of the tail to snout) or standard length (i.e., fork to snout), whichever is desired. Record the length.

9.4.3.3.7 Weigh a polypropylene sample jar on the balance and record the mass. Tare the balance and dissect the desired tissue(s) from the fish (e.g., digestive tract, fillet, liver, etc.). Record the sex of the fish if identifiable. Place the target tissue into the jar, record the mass and close the jar.

9.4.3.3.8 Repeat steps 9.4.3.3.4 through 9.4.3.3.7 for the remaining fish to be sampled. If samples are to be pooled, tissues may be deposited into the same

sample jar, taring the balance each time or subtracting the mass of the jar and other tissues post-hoc.

9.4.3.3.9 Close or cover the DB.

9.4.3.3.10 Store samples at -20°C or in 70-100% ethanol filtered through a pore-size of 1 µm or smaller at 4°C until processing and analysis. Note that samples should not undergo more than one freeze-thaw cycle or be stored at temperatures lower than -20°C (Section 12.2).

10.0 Reagents and Standards

10.1 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 4.2.3.1) and covered (Section 4.2.3.3) until use.

10.2 Ethanol used to preserve tissue samples must be filtered through a pore-size of 1 µm or smaller prior to use.

10.3 The DB should be created by the laboratory by adding 250 mL of MAG water to a clean (Section 4.2.4.1) sampling jar. One DB should be prepared for every set of samples dissected or collected (in the case of whole fish) at same time.

11.0 Quality Control

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.

11.1 Dissection Blank (DB) – A DB must be included with each set of samples dissected at the same time and analyzed to assess contamination during dissection, shipping and storage. Microplastic levels in the DB must be below the MRL; if not, the batch of samples associated with the DB must be flagged accordingly.

11.2 Trip Blank – A Trip Blank must be evaluated before the sampling event if a different type of sampling container is used from those listed in Sections 8 and 9. The use of a Trip Blank is optional if recommended sampling jars are used (Sections 8 and 9). If Trip Blanks are used, they do not need to be analyzed unless the DB shows evidence of contamination. The Trip Blank may be analyzed to determine if contamination occurred during shipping or travel.

11.3 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 and test the rinse 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 monthly. If sampling occurs less frequently than once a month, conduct this verification immediately prior to each sampling event (Section 4.2.2.8).

12.0 Sample Preservation and Storage

12.1 To prevent tissue decay, samples must be stored at low temperature (i.e., 4 ± 2 °C) immediately upon collection. It is highly recommended that bivalves are shucked and fish are dissected and tissues frozen or preserved (i.e., ethanol filtered through a pore-size of 1 µm or smaller) as soon as possible to prevent specimen loss from eventual decay.

12.2 It is preferable that samples are dissected and processed before any freezing occurs. If this is not possible, samples must not undergo more than one freeze-thaw cycle prior to analysis. Frozen storage at -20 °C prior to shucking and/or dissection will preserve biological tissue but is not recommended given the potential for freeze-thaw cycles to fragment any microplastic particles further or contribute to particle loss. Samples should never be frozen at temperatures lower than -20°C.

12.3 Trip Blanks may 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 LRFBs.

13.0 Field Data Reporting Requirements

13.1 Data to be reported when sampling aquatic biota for microplastics analysis are listed in the table below.

Data Type	**Description**
Location	Degrees of latitude and longitude expressed in decimal degrees to 5 decimal places.
Date	The date the sample was collected (i.e., yyyy-mm-dd).
Time	The time the sample was collected (i.e., hh:mm:ss).
Weather Conditions	Description of the weather conditions during sampling (e.g., mostly sunny, light winds ~10 mph).
Sampling Gear Type	Description of the sampling device(s) used to collect biota (e.g., seine net).
Field Crew Gear	Picture (optional) and description of gear used by field crews and apparel worn.
Habitat Type	Description of the habitat where organisms were collected.
Species	The name of the species targeted for collection for microplastics analysis.
Organism Morphometrics	Description of the condition and morphometrics of collected organisms (e.g., age, mass, length, sex).
Analyzed Tissue Mass	Mass (wet weight) of the tissues analyzed for microplastics analysis.
Dissection Blank Type	Description of the type of blank used (e.g., aliquot of microplastics analysis grade water in a sampling jar, wetted filter in petri dish).
Replicate Number	If replicate samples are collected, the replicate number of the sample.

14.0 Waste Management

14.1 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 the landfill.

15.0 References

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Standard Operating Procedures for the Collection of Aquatic Biota Samples for Microplastics Analysis, Great Lakes Addendum

Primary reference SOP:
Southern California Coastal Water Research Project Authority Working Group (SCCWRP), 2023. Standard Operating Procedures for the Collection of Aquatic Biota Samples for Microplastics Analysis, v.4, 15 p.

Medium introduction

The analysis protocol and many of the collection techniques described in the primary reference SOP (SCCWRP, 2023) are applicable to the Great Lakes, but there are some important differences in sampling methods and species that are described here. The primary aquatic organisms of interest for the Great Lakes are fish and benthic invertebrates. The latter were prioritized given that microplastics accumulate in sediments and exposures in this habitat will likely be higher than those of pelagic habitats. The primary reference SOP and this addendum would enable long-term, consistent monitoring of microplastics in organisms to understand spatial and temporal trends. Offshore sampling of biota can take advantage of long-running biota monitoring programs (e.g., USEPA Great Lakes Monitoring [Barbiero et al., 2018]). Several investigators have performed prior studies of microplastic exposure, ingestion, and risk in biota using a variety of sampling methods and organisms. Studies on microplastics in fish in the Great Lakes include work by Munno et al. (2022). Studies of microplastic ingestion by quagga mussels, the primary Great Lakes filter feeder, have been published by Pedersen et al. (2020). Earn et al. (2021) published a recent review, and Hataley et al. (2023) performed a risk assessment. Future collection of aquatic biota samples for microplastics analysis would build on this prior work.

Modifications to primary reference SOP by section

1.0 Scope and Application

No additions or modifications.

2.0 Summary of Method

No additions or modifications.

3.0 Definitions

No additions or modifications.

4.0 Interferences

No additions or modifications.

5.0 Safety

No additions or modifications.

6.0 Taxa Selection

The general principles of this SOP section (see SCCWRP, 2023) are applicable, but recommended taxa are modified below

- What is the exposure of pelagic organisms to microplastics?
    - Recommended taxa: forage fish (e.g., alewife, bloater, sculpin, and rainbow smelt)

- What is the exposure of sediment-dwelling organisms to microplastics?
    - Recommended taxa: quagga mussels, Asian clams, demersal fish (e.g., round goby)
- What is human exposure to microplastics through subsistence fishing?
    - Recommended taxa: sport fish (e.g., yellow perch, walleye, lake trout)

7.0 Sample Size and Tissue Mass Recommendations

No additions or modifications.

8.0 Bivalve Collection: Equipment, Supplies, and Procedures

8.1 and 8.2 No additions or modifications.

8.3 Oyster and/or mussel collection.
Collection methods specific to oysters are not applicable to the Great Lakes.

8.4 Collecting clams for microplastics analysis.
Benthic invertebrates are currently collected as part of several offshore monitoring programs in the Great Lakes. Samples are typically recovered using Ponar samplers or box corers. Biota acquired in this way can be collected for microplastic analysis using plastic-free sampling approaches described in the primary reference SOP. The clam sampling protocol described in the SOP is oriented toward sampling in intertidal zones. For the Great Lakes, sampling of unionid mussels, quagga mussels, or other benthic invertebrates by hand via wading, snorkeling, or diving has been performed successfully following approaches such as those described by Keretz et al. (2023) or Bryan et al. (2013). Many unionid species are threatened or endangered in the Great Lakes, so these species should not be targeted for routine monitoring of microplastics.

8.5 Cleaning and shucking bivalves (i.e., oysters, mussels, clams).
Processing methods specific to oysters in this section of the primary reference SOP are not applicable to the Great Lakes.

9.0 Fish Collection: Equipment, Supplies, and Procedures

No additions or modifications.

10.0 Reagents and Standards

No additions or modifications.

11.0 Quality Control

No additions or modifications.

12.0 Sample Preservation and Storage

No additions or modifications.

13.0 Field Data Reporting Requirements

No additions or modifications.

14.0 Waste Management

No additions or modifications.

15.0 References

Additional references cited include the following:

Barbiero, R.P., Lesht, B.M., Hinchey, E.K., and Nettesheim, T.G., 2018. A brief history of the US EPA Great Lakes National Program Office's water quality survey. Journal of Great Lakes Research, 44(4), pp.539-546.

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Earn, A., Bucci, K., and Rochman, C.M., 2021. A systematic review of the literature on plastic pollution in the Laurentian Great Lakes and its effects on freshwater biota. Journal of Great Lakes Research, 47(1), pp.120-133.

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Pedersen, A.F., Gopalakrishnan, K., Boegehold, A.G., Peraino, N.J., Westrick, J.A., and Kashian, D.R., 2020. Microplastic ingestion by quagga mussels, *Dreissena bugensis*, and its effects on physiological processes. Environmental Pollution, 260, p.113964.

Standard Operating Procedures for the Collection of Sediment Samples for Microplastics Analysis

Prepared by

Southern California Coastal Water Research Project Authority

27 June 2024

1.0 Scope and Application

1.1 This method is for the collection of sediment samples for the determination of the concentration and composition of microplastics, as defined by the State of California 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.

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).

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.

2.0 Summary of Method

This method is adapted from commonly used sediment sampling techniques for other assessment types (e.g., chemical contaminants, benthic macroinvertebrates, etc.). This method describes the collection of sediment for microplastic analysis, providing the option between a hand coring device and a Van Veen grab sampler. The choice of sampling device is dependent on sampling and logistical conditions (i.e., non-wadeable versus wadeable access). Both techniques target the top five centimeters of sediment. This sampling depth typically represents the greatest concentration of microplastic particles by count (Martin et al., 2017; Fan et al., 2019; Zheng et al., 2020; Kukkola et al., 2022; Yu et al., 2023), the depth at which many benthic organisms reside, and is consistent with the vast majority of sediment sampling methodologies for the determination of microplastics around the world (JRC 2014; BASEMAN 2019; U.S. EPA 2020; Bight Regional Monitoring Program 2023).

3.0 Definitions

Field blank (FB) – An aliquot of MAG (Microplastics Analysis Grade, see definition below) water that is placed in a sample container or a wetted filter placed in a petri dish. The FB is treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, storage, preservation, and all analytical procedures. 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 sent out for each sampling event and analyzed alongside field collected samples. The volume of the FB must be similar to that of actual samples collected and processed by this method. 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. Analysis results from the FB must be reported alongside analysis results from collected samples. If the FB particle count is greater than the Method Reporting Limit (see definition below), all associated field collected samples (i.e., collected during the same sampling event) must be flagged accordingly. 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 are 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).

Microplastics – Solid[1] polymeric materials[2] to which 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 1 µm or smaller. Filters must be of an appropriate material that does not shed particles (Section 4.2 and 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).

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 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 sample collection period. A Trip Blank must be

[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.

evaluated prior to the sampling event if different sampling containers from those listed in Section 6 are used.

4.0 Interferences

4.1 Physical Interferences

4.1.1 Preventing sediment samples from becoming contaminated during collection can be one of the greatest difficulties encountered in quantifying microplastics in sediment 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 sediment 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, synthetic ropes and lines on sampling vessels, apparel worn by field personnel, carpets, and furniture), 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 must use as much plastic-free equipment 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 4.2.1.3 to 4.2.1.7.

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 hard plastic tubing (e.g., Tygon® or clear PVC tubing) to dispense water used to make MAG water is acceptable.

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 coring device cap is made from polyvinyl chloride. It has minimal contamination potential but should be thoroughly cleaned and evaluated for shedding according to procedures described in Section 8 – Quality Control.

4.2.2.7 The coring device may be made from clear acrylic to facilitate the inspection of sample condition and penetration depth when sampling. Acrylic (i.e., polymethyl methacrylate) is rarely detected in environmental samples analyzed for microplastics and has minimal contamination potential. However, it should be thoroughly cleaned and evaluated for shedding according to procedures described in Section 8 – Quality Control.

4.2.2.8 If plastic materials are used, inspect their integrity. FBs exist to help account for any procedural contamination from plastics during sample collection. Examples of plastics commonly used in microplastics sample collection that are acceptable as they do not shed polymer particles are listed in Sections 4.2.2.3 and 4.2.2.7.

4.2.2.9 All plastic apparati shall be evaluated immediately prior to each sampling event or on monthly basis, whichever occurs less frequently, for potential to shed microplastics by 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 material 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 field expedition, 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. Ash heavy-duty foil only, as the lightweight foil will disintegrate at high temperatures. Discard foil after use.

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

4.2.5.4 When feasible, cover all equipment with pre-kilned, 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 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.

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

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

6.0 Equipment and Supplies

6.1 Equipment and supplies for both wadeable and non-wadeable sampling approaches 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.

6.1.1 A hand coring device is recommended for wadeable sampling sites where the use of a Van Veen grab sampler is impractical or not desired.

6.1.2 In wadeable conditions where sediment is not covered by water (e.g., mudflats at low tide), a metal or acrylic cylinder at least 5 cm in height and without caps may be used.

6.1.3 If sediment is covered by water at the time of sampling, the core must be capped on both ends before retrieving it through the water column. Any coring device may be used so long as the barrel is marked at the appropriate sampling depth (i.e., 5 cm). The barrel must be made from metal (e.g., steel, aluminum) or acrylic.

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	47 mm diameter, Sterlitech Polycarbonate Track Etch (PCTE) membrane filters, Catalog no. PCT1047100
Sample Collection	
Nitrile gloves	Kimtech™ Purple Nitrile™ Exam Gloves (Product code #55083)
Pre-kilned, heavy-duty aluminum foil	-
Laboratory labelling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or LDPE)	VWR no. 16651-904
Non-wadeable conditions: Van Veen grab sampler OR *Wadeable* conditions, no water: 2 in. diameter x 2 in. height metal cylinder OR *Wadeable* conditions, with water: Hand coring device with coring device cap	Wildco 1728-G30 grab dredge, 6" x 6" sampling area, Cole-Palmer, Item #UX-05471-10 Amazon – "Round Cake Rings Mold, 2-Inch Mini Cake and Pastry Ring, Stainless Steel" See Section 6.2 for coring device materials and construction
Metal ruler	-
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 11.2 for sampling jar requirements.
Metal scoop (Van Veen method) OR Metal spoon (Hand coring device method)	-
Metal spade	-
Wide metal spatula (optional)	Grainger - Stainless Steel Blade - Item #45GL08
Bubble wrap	-
Cooler or heavy-duty storage container	-

6.2 Equipment and supplies for the construction of a metal coring device are listed in the table below.

Item	**Suggested Materials**
Core barrel	18 in. long, ~3 in. diameter metal or acrylic tube
Pipe cap with stainless steel tightening clamp	3 in. PVC Flexible Pipe Cap with Stainless Steel Clamps, Home Depot, Model #E03713
Riser clamp (optional, see Section 6.2.4)	3 in. Riser Clamp in Galvanized Steel, Home Depot, Model #03CLRSGE
Wing nuts (optional, see Section 6.2.4)	Select size based on size of riser clamp bolts
Metal spade or shovel (optional, see Section 6.2.4)	-
Saw appropriate for cutting metal or acrylic	-
Power Drill	-

Rubber Stopper	No. 3 size (18 mm bottom diameter) or smaller
Rasp file, Dremel tool, etc.	-
Duct tape or other waterproof tape	-

6.2.1 If the pipe is longer than 18” trim to appropriate length. Eighteen inches is a recommendation, but a few inches longer or shorter is acceptable if more ergonomically comfortable.

6.2.2 If using an acrylic tube, use a file, Dremel tool or other preferred device, to cut a bevel at one end of the tube, which will become the bottom (Figure 1) and will aid in penetrating the sediment.

6.2.3 Mark the depth guide for inserting the core into the sediment. Measure 5 cm up from the bottom of the core and wrap weatherproof tape or indelible marker around the core to mark it.

6.2.4 If there is a need to remove excess water from the coring device during sampling (e.g., sampling sediment below standing water), drill a hole in one side of the coring device barrel 1-5 cm above the depth guide, depending on the desired depth of penetration (see Section 9.2.5.1). The diameter of the hole should be larger enough to fit the rubber stopper securely. Insert the rubber stopper.

6.2.5 If a handle for the coring device is desired, affix the riser clamp approximately 12-15” from the bottom of the core to create a handle for inserting and removing the core (Figure 2). Replace the hex nuts with wing nuts for ease of adjustment by hand. Alternatively, the core may be extracted using a metal spade or shovel (See Section 9.2.5).

6.2.6 Ensure rubber cap fits the coring device.

6.2.7 Inspect the coring device for any areas where plastic particles may be likely to shed (e.g., drilled holes). If necessary, use a file or sandpaper to remove any rough edges prone to shedding. Before each sampling event, the coring device must be evaluated for shedding as described in Section 4.2.2.7 and cleaned before each field expedition according to Section 4.2.5.

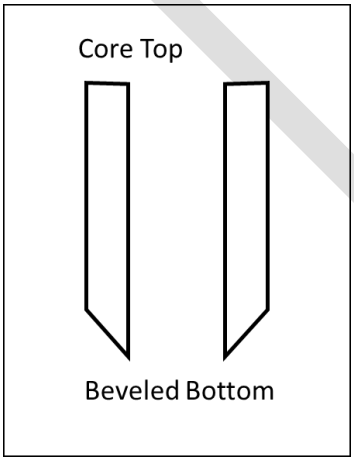

Figure 1. Diagram of beveled core bottom for better sediment penetration.

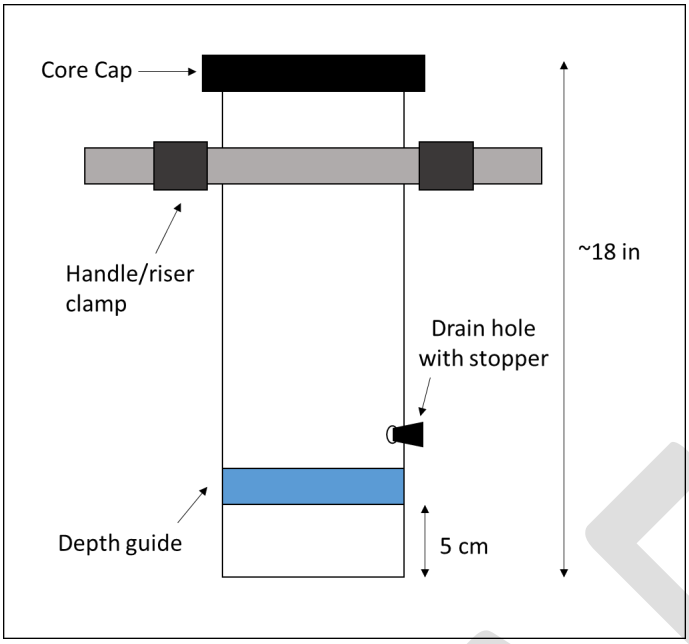

Figure 2. Basic diagram of coring device.

7.0 Reagents and Standards

7.1 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 4.2.5.4) until use.

7.2 The FB should be created by the laboratory by adding 250 mL of MAG water to a clean (Section 4.2.5.1) sampling jar. One FB should be prepared for every set of samples collected at the same site and same time.

8.0 Quality Control

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.

8.1 Field Blank (FB) – A FB must be included with each sampling event and analyzed to assess contamination during shipping and storage. 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.

8.2 Trip Blank – A Trip Blank must be evaluated before the sampling event if a different type of sampling container is used from those listed in Section 6. The use of a Trip Blank is optional if recommended sampling jars are used (Section 6). If Trip Blanks are used, they do not need to be analyzed unless the FB shows evidence of contamination. The Trip Blank may be analyzed to determine if contamination occurred during shipping or travel.

8.3 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 11.2). Cover the opening of the jar with pre-kilned heavy-duty foil and store according to Section 11.1. The sample may then be shipped (Section 11.2) 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 immediately prior to each sampling event or monthly, whichever occurs less frequently (Section 4.2.2.9).

9.0 Procedure

The following procedures assume that all equipment and supplies have been cleaned according to previously described requirements (see Section 4.2.5). Sampling approaches for using a Van Veen grab sampler and a metal coring device are described for sampling non-wadeable and wadeable environments, respectively.

While it is generally recommended that at least 100 g of sediment are collected for microplastic analysis, determination of sediment 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 amount of sediment required for analysis to ensure detectable amounts of microplastics.

9.1 Sampling via Van Veen grab sampler in non-wadeable conditions.

9.1.1 Rinse the Van Veen grab sampler with ambient water three times, followed by MAG water three times.

9.1.2 Deploy the Van Veen to collect sediment.

9.1.3 Upon retrieval of the Van Veen, open the FB jar. Leave the open jar as close as possible to where the sediment will be collected. Place the FB jar lid face down on a clean piece of pre-kilned, heavy-duty aluminum foil. Alternatively, the FB jar lid may be wrapped in pre-kilned, heavy-duty aluminum foil and set aside.

9.1.4 Rinse the clean metal scoop or spade three times with MAG water.

9.1.5 Open the Van Veen doors to inspect the condition of the sample to ensure it meets acceptability criteria (Section 10.2). Drain off the overlying water. Measure and record the penetration depth by inserting a ruler vertically along the grab midline.

9.1.6 Use the metal scoop or spade to collect sediment from the top 5 cm of sediment. Fill the sampling jar with the desired amount of sediment. (A minimum of ~100 g wet weight or ~500 mL is recommended for most analytical procedures.) Cover the jar with pre-kilned, heavy-duty aluminum foil. Rinse the lid with MAG water and screw on the lid tightly. If sediment is spilled on the outside of the jar, it may be cleaned with a gloved finger or cellulose wipe such as paper towel.

9.1.7 Immediately after the sediment sample has been collected, remove the FB lid from the foil and rinse it three times with MAG water. Cover the jar opening with pre-kilned, heavy-duty aluminum foil. Rinse the lid with MAG water and screw on the lid tightly.

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

9.2 Sampling via hand coring device in wadeable conditions.

9.2.1 Identify an undisturbed ~5 x 5 ft area without vegetation, taking care not to step in the sampling area.

9.2.2 Rinse the coring device with ambient water three times, followed by MAG water three times.

9.2.2.1 If water is over the sampling area, rinse the coring device caps with ambient water three times, followed by MAG water three times.

9.2.3 Open the FB jar. Leave the open jar as close as possible to where the sediment will be collected. Wrap the lid in pre-kilned, heavy-duty aluminum foil and set aside.

9.2.4 Push the coring device into the sediment to a depth of at least 5 cm. This may be the top of the coring device if using a 2-inch metal cylinder or to the depth guide marked on the outside of the core barrel.

9.2.4.1 If water is over the sampling area, affix the first coring device cap to the top of the coring device after pushing it into the sediment.

9.2.5 Use the metal spade, shovel, or a gloved hand to remove the sediment from the side of the inserted coring device.

9.2.6 Slide a gloved hand, wide metal spatula, or second coring cap under the core barrel to keep the sediment from falling out of the bottom of the core, using care not to push the sediment up and out of the core.

9.2.7 Extract the coring device from the sediment by pulling upward or using a metal spade or shovel to pry the core from the sediment. Inspect the core to ensure it meets condition and depth requirements (Section 10.3), removing the top coring device cap if necessary. Hold the coring device over the sampling jar in case the sediment falls unexpectantly and remove the coring device caps, one at a time. Use the metal spatula to push the sediment into the sample jar. Cover the lid of the sample and FB jars with pre-kilned, heavy-duty aluminum foil.

9.2.7.1 If the coring device was inserted to a depth greater than 5 cm, extrude the excess sediment from the coring device by allowing it to slowly fall out the bottom of the coring device until the top of core is at the 5 cm depth guide.

9.2.7.2 If excess water is trapped inside the coring device, it may be drained by removing the plug on the side of the coring device until ~1 cm of water remains. Alternatively, water may be deposited into the sample jar with the sediment, but the sample jar must be an adequate volume to ensure 100 g wet weight of sediment may be collected.

9.2.8 Repeat steps described in Sections 9.2.1 to 9.2.5 until the desired amount of sediment has been collected, depositing each core into the same sample jar. (A minimum of ~100 g wet weight or ~500 mL is recommended for most analytical procedures.) Cores should be taken ~6 inches to 1.5 ft apart within the sampling area where the sediment has not been previously disturbed.

9.2.9 Label both the sample and FB jars, wrap in bubble wrap, and store in a secondary container (e.g., cooler) for protection during transport.

10.0 Sampling Acceptability Criteria

10.1 Upon retrieval of the grab or core, the acceptability of the sample must be determined. Acceptability criteria are adapted from the Southern California Bight Regional Monitoring Sediment Quality Assessment Field Operations Manual.

10.2 Acceptability for grab samples collected using a Van Veen under non-wadable conditions is based upon two characteristics: sample condition and depth of penetration.

10.2.1 Sample condition is judged using criteria for surface disturbance, leakage, canting, and washing (Figure 3). An acceptable sample condition is characterized by an even surface with minimal disturbance and little or no leakage of the overlying water. Heavily canted samples are unacceptable. Samples with a large amount of "humping" along the midline of the grab, which indicates washing of the sample during retrieval, are also unacceptable. While some humping will be evident in samples taken from firm sediment where penetration has been poor, this can be due to the closing action of the grab and is not necessarily evidence of unacceptable washing.

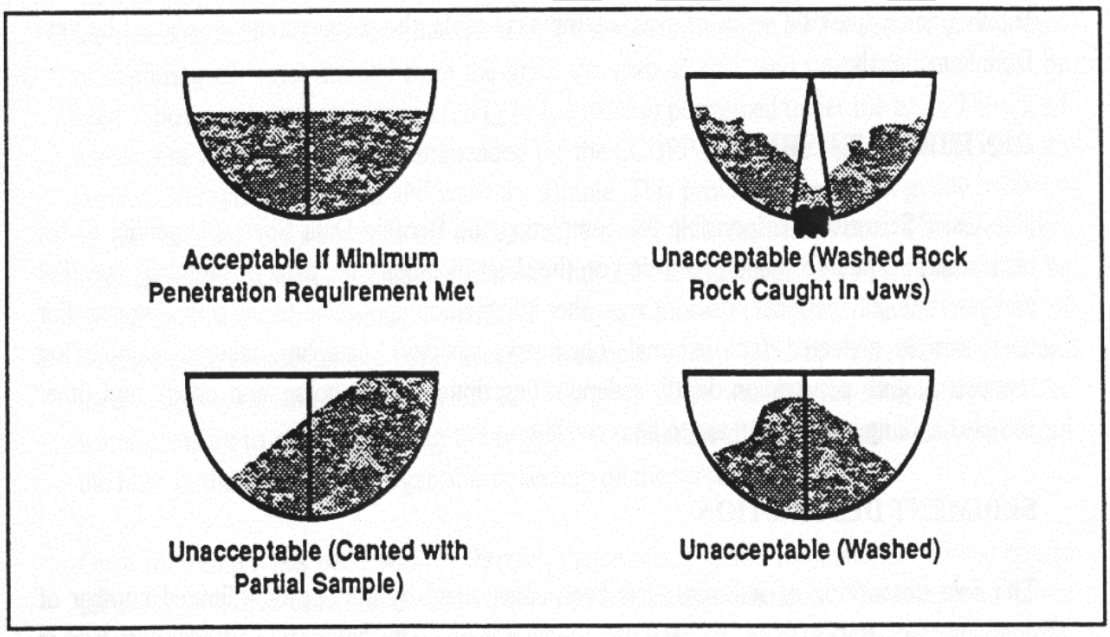

Figure 3. Examples of acceptable and unacceptable grab sample conditions.

10.2.2 Sample condition may be recorded by taking a photo.

10.2.3 The depth of penetration is determined by insertion of a metal ruler vertically along the grab midline and measuring to the nearest 0.5 cm. Sediment penetration depth for all grabs must be sufficient to sample the top 5 cm of sediment without touching the bottom of the Van Veen. In habitats where sediments are unusually soft (*e.g.,* some estuary muds), it may be necessary to remove the lead weights to prevent over-topping the grab.

10.3 Acceptability for samples collected using a hand coring device under wadable conditions is based upon two characteristics: sample condition and depth of penetration.

10.3.1 Sample condition is judged based on the sediment core immediately after extraction and prior to depositing sediment into the sample jar. If the core is incomplete (i.e., there is missing sediment from the core or gaps within the core due to the coring device hitting bedrock, excess gravel or cobble falling out of the core, or extraction mishandling), then another attempt should be made to collect an intact core. If the top or bottom layer of the core is significantly disturbed during extraction, the core should be rejected, and another attempt should be made to collect a new core within the sampling area. If it is not possible to collect an undisturbed sample after multiple attempts, details regarding the degree of disturbance should be recorded.

10.3.2 The depth of penetration is determined using the depth guide marked on the core barrel or the coring device itself when appropriately sized (i.e., 5 cm in height). Sediment penetration depth must be at least 5 cm. If the penetration depth is less than 5 cm, the core should be rejected and reattempted in a new location within the sampling area.

11.0 Sample Preservation and Storage

11.1 Samples must be stored at low temperature (e.g., 4 ± 2 °C), to prevent bacterial growth. 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 pre-kilned, heavy-duty aluminum foil. It is not recommended that samples be frozen, but, if necessary, one freeze thaw cycle is acceptable (-20°C).

11.2 Glass containers with non-plastic lid liners (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. Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts 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. Shipping samples on ice (< 6 °C) is preferred, but samples may be shipped at room temperature.

11.3 Trip Blanks may 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 LRFBs.

11.4 Field Blanks must accompany each set of sample containers taken from the laboratory to the sampling site and back. At the beginning of the sampling event and for its duration, keep the FB container open at the site while collecting the sample. 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 sediment for microplastics analysis are listed in the table below.

Data Type	Description

Location	Degrees of latitude and longitude expressed in decimal degrees to 5 decimal places.
Date	The date the sample was collected (i.e., yyyy-mm-dd).
Time	The time the sample was collected (i.e., hh:mm:ss).
Sampling Conditions	Was the sampling site wadable or non-wadable?
Water Conditions	General description of water appearance (e.g., turbidity, color)
Weather Conditions	Description of the weather conditions during sampling (e.g., mostly sunny, light winds ~10 mph)
Water Present	Was there standing water over the sediment to be sampled?
Sampling Gear Type	Description of the sampling device used (e.g., Van Veen)
Field Crew Gear	Picture (optional) and description of gear used by field crews and apparel worn
Sampling Attempts	Number of sampling attempts made before acceptable sample obtained (see Section 10)
Sediment Condition Description	Picture (optional) and qualitative description of the sediment (e.g., visual texture, degree of consolidation)
Field Blank Type	Description of the type of field blank used (e.g., aliquot of microplastics analysis grade water in a sampling jar, wetted filter in petri dish)
Replicate Number	If replicate samples are collected, the replicate number of the sample

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.

14.0 References

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Standard Operating Procedures for the Collection of Sediment Samples for Microplastics Analysis, Great Lakes Addendum

23 July 2024

Primary Reference SOP:
Southern California Coastal Water Research Project Authority Working Group (SCCWRP), Nov 2023 draft. Standard Operating Procedures for the Collection of Sediment Samples for Microplastics Analysis, v.4, 15 p.

Medium Introduction:
Microplastics in sediment may be of concern for benthic organisms (Koelmans et al., 2023), particularly in the biologically active zone of the surface sediment. Continued monitoring through standardized sampling protocols will enable the assessment of temporal trends in exposure risk. This protocol is intended to support sediment monitoring that evaluates exposure risk for benthic organisms and is not to assess loading rates or to extrapolate temporal trends from a single sampling event. There are several different methods for sampling settling microplastics to quantify loading rates, which include deploying sediment traps. Sampling methods should be selected to correspond to the monitoring objective and thus, the monitoring objective should be considered when choosing a sampling method. This monitoring could be supplemented by research studies to understand the transport, fate, and variability of microplastic temporal and spatial patterns and processes over weekly to multi-decadal time scales.

Modifications to Reference SOP by Section:

1.0 Scope and Application
No modifications. Note the addition of details below related to sediment coring in deep water (Section 9).

2.0 Summary of Method
No additions or modifications.

3.0 Definitions
No additions or modifications.

4.0 Interferences
No additions or modifications.

5.0 Safety
The primary reference SOP (SCCWRP, 2023) does not give specific guidance for sampling from vessels, but appropriate safety protocols for this type of sampling should be followed, especially in large tributaries or nearshore and open waters of the Great Lakes.

6.0 Equipment and Supplies
No modifications. Note that the equipment list in the primary reference SOP is oriented toward core sampling on foot and by hand under wadable conditions, or a Van Veen grab in deeper water. In addition to the sampling gear described therein, other types of sampling equipment that are common for sampling sediment in the Great Lakes and their tributaries can be used as long as only the top 5 cm are collected and the surface area of the sample is reported.

7.0 Reagents and Standards

No additions or modifications.

8.0 Quality Control

No additions or modifications.

9.0 Procedure

The primary reference SOP (SCCWRP, 2023) describes approaches for collecting samples directly via coring while wading and in deeper water by Van Veen grab sampler. In lakes and tributaries of the Great Lakes, any grab or core sampler could be used that is appropriate to the size of the system or vessel and the composition of the sediments as long as only the full top 5 cm and the sediment-water interface is collected and the surface area of the sample is recorded. Consistency in sampling depth and methods is critical to achieve comparability in exposure risk assessment among sampling campaigns and monitoring programs.

Site selection is not discussed in detail beyond avoiding sites with vegetation in shallow water. For Great Lakes sampling programs, the sampling locations should be selected with consideration of proximity to microplastic sources, the likely presence of sediment that can be recovered by the sampling device chosen (e.g., avoiding hard substrates and gravel), and the lake processes and phenomena that may influence microplastic concentrations (e.g., waves, currents, ice, sediment accumulation rates, and other chemical contaminants in sediment).

The primary reference SOP (SCCWRP, 2023) provides instructions on cleaning sampling equipment that must be followed. Care should be taken with each of the devices listed in Section 6.0 to avoid sample contamination.

10.0 Sampling Acceptability Criteria

No additions or modifications.

11.0 Sample Preservation and Storage

No modifications.

Chemical and porewater gradients that are of concern in some sediment coring studies would be less of an issue with microplastics due to their relatively refractory nature. If sediment cores are not processed in the field, they should be securely capped, have overlying water drained as described in the primary reference SOP, and be kept upright and with minimal vibration during transport from the sampling vessel to the laboratory. This would minimize vertical mixing that could occur if saturated cores were laid on their sides or allowed to vibrate and roll during transport.

12.0 Field Data Reporting Requirements

No modifications.

13.0 Waste Management

No additions or modifications.

14.0 References

Additional references cited include the following:

Koelmans, A. A., Redondo-Hasselerharm, P. E., Mohamed Nor, N. H., & Gouin, T. (2023). On the probability of ecological risks from microplastics in the Laurentian Great lakes. *Environmental Pollution, 325*, 121445. https://doi.org/10.1016/j.envpol.2023.121445