Standard Operating Procedures for the Collection of Samples for Microplastics Analysis Part 1: Surface Sediment and Aquatic Biota

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SOUTHERN CALIFORNIA COASTAL WATER RESEARCH PROJECT

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EXECUTIVE SUMMARY

The California Statewide Microplastics Strategy emphasizes the need to better understand the extent and magnitude of microplastics contamination in the coastal environment to inform potential solutions and management decisions. Consequently, a Statewide Plastics Monitoring Strategy and Multi-Year Plan is under development to build a statewide monitoring network by leveraging existing monitoring programs across the state. Method standardization is a key part of this plan to ensure that samples are collected and analyzed in a consistent manner and generate meaningful, comparable, and high-quality data. Analytical methods for the analysis of microplastics in drinking water have already been adopted by the State Water Board, and methods for the extraction and analysis of microplastics from sediment, biological tissues, and water containing organic material have undergone a multi-laboratory performance evaluation in preparation for potential adoption. Efforts to evaluate collection methods for drinking water are currently underway, but there are no standardized sample collection methods available for other environmental media. Though sample collection for microplastics analysis is similar to methods used for other contaminants, there are some key differences to mitigate sample contamination and ensure that samples are accurate, precise, and representative. Therefore, the goal of this project is to produce standardized collection methods for surface sediments, aquatic biota, and stormwater flows. On March 8-9th, 2023, technical experts across a variety of sectors attended a two-day in-person workshop at the Southern California Coastal Water Research Project headquarters in Costa Mesa, California to select collection methods for microplastics ready for evaluation and create a plan for developing standardized protocols. For each matrix, experts identified method parameters most likely to influence microplastics analysis results and designed and prioritized evaluation studies for each. Sampling depth and the sampling device used were identified as the most important variables for surface sediment collection whereas taxa selection, sample size, and the total biomass amount were identified as the most important for aquatic biota. Experts agreed that there was enough reliable data already available in the scientific literature to proceed with drafting standard operating procedures without experimental evaluation. In addition to providing specific guidance for the variables identified above, each protocol also details specific guidance for the mitigation and characterization of background contamination (i.e., microplastic particles inadvertently introduced during sample collection) that may occur during sampling, a well-known challenge in microplastic research. Experts agreed that sample collection methods for stormwater flows required experimental evaluation studies prior to developing standardized protocols. Therefore, the evaluation of sample collection methods for stormwater flows will be presented in a second technical report (Part 2).

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INTRODUCTION

The California Statewide Microplastics Strategy outlines a two-track approach to take immediate action to mitigate plastic pollution while simultaneously conducting research to inform future management actions (OPC 2022). The research-track describes the need to initiate a statewide monitoring network by leveraging existing monitoring programs such as the Surface Water Ambient Monitoring Program (SWAMP), the San Francisco Bay Regional Monitoring Program, and the Southern California Bight Regional Program to gain a comprehensive view of microplastic contamination across the state. Existing programs can provide infrastructure for the coordinated collection and analysis of samples. This vision is currently being enacted through the development of a Statewide Plastics Monitoring Strategy and Multi-Year Plan led by the San Francisco Estuary Institute (SFEI). This plan will be informed by a federal and state advisory group, technical experts, tribes, and the public (SFEI 2024). Standardized methodologies for the collection and analysis of microplastics samples represent a critical part of this plan to ensure that the data collected are useful, comparable, and meet essential data quality objectives. Analytical methods for microplastics quantification and characterization have been evaluated for drinking water, water containing organic material (e.g., ambient water), sediment, and biota (De Frond et al. 2022; Thornton Hampton et al. 2023), and standardized protocols for the analysis of microplastics in drinking water have been adopted by the California State Water Resources Control Board (SWRCB 2022a; 2002b). Laboratories may also receive accreditation for these methods through the Environmental Laboratory Accreditation Program (ELAP) (Wong & Weisberg 2024). Efforts to evaluate and standardize collection methods for drinking water are currently underway as outlined in the Policy Handbook Establishing a Standard Method of Testing and Reporting of Microplastics in Drinking Water (SWRCB 2022c).

Collection methods for matrices beyond drinking water have not been evaluated or standardized. This represents a critical need for microplastic monitoring efforts in aquatic ecosystems. Sample collection for microplastics is partly unique from other contaminants due to the challenges associated with the mitigation and characterization of background contamination. For example, microplastics are ubiquitous in the ambient environment, and samples may be contaminated during collection from aerial deposition, equipment shedding, or synthetic clothing (Brander et al. 2020). Specialized procedures (e.g., regular and thorough cleaning of equipment with microplastic-free water, wearing of cotton lab coats or clothing) must be implemented to mitigate contamination as much as possible. However, it is often impractical, if not impossible, to completely eliminate background contamination throughout the collection and analytical process. Background contamination rates must be quantified and characterized to establish analytical limits of detection and quantification and ensure

confidence in the accuracy of results (Lao & Wong 2023). A series of methodological blanks are used to track background contamination throughout sample collection and analysis. Data collected from these blanks may also be used to identify and eliminate potential sources of contamination if necessary.

The heterogenous nature of particulates also requires special consideration to ensure that collected samples are accurate, precise, and representative (Brander et al. 2020). As insoluble particles, microplastics are not evenly distributed within a matrix and may aggregate or disperse in unpredictable and complex ways (He et al. 2021; Li et al. 2023; Li et al. 2024). This behavior depends on the matrix and particle characteristics such as morphology, size, weathering, and density and may cause high levels of variability among replicate samples. Variability challenges may be overcome by increasing the amount of material or number of replicate samples collected, but there are practical and logistical limitations to be considered (e.g., It is not typically feasible to transport hundreds of liters of water). Many factors including, but not limited to, the matrix, the habitat, the predicted level of contamination and variability, the sampling device, the extraction method, the analytical method, and the background contamination rate inform the number of samples, and the amount of material collected. Most importantly, the sampling design and collection method used must consider the acceptable level of variability for the research or management questions to be addressed.

Background contamination and challenges associated with heterogeneity and high variability cut across matrices, but there are also matrix-specific considerations for sample collection. Sediment sampling techniques for any contaminant differ based on logistics and the research or management question at hand. Microplastics are no exception. For example, a sampling depth of 5 cm at one site may not be directly comparable to another due to differences in deposition rates, and the vertical distribution of microplastics in the sediment may be influenced by particle characteristics such as size (Waldschläger et al. 2022). There is often also a need to use different sampling devices for different habitats and sampling situations. A coring device may be used in areas where the sediment is exposed or in wadable conditions whereas a Van Veen sampler must be used in deeper waters. There are a different set of considerations for aquatic biota. Different taxa or tissues may be targeted for sample collection for microplastic analysis. These samples will likely exhibit different concentrations of microplastics per tissue mass due to inherit biological differences such as habitat and life history (McNeish et al. 2018; Piarulli et al. 2020). Tissue isolation may also require the use of additional background contamination precautions and procedural blanks during dissection.

There is a need to develop matrix-specific guidance and collection procedures to ensure that samples are collected in a manner that will generate accurate and reliable results to address the research and management questions at hand and inform the California Statewide Plastics

Monitoring Strategy. The goal of this project was to produce standardized sample collection protocols for monitoring environmental microplastics in stormwater flows, sediment, and biota. To do this, a group of international experts in microplastic sample collection and analysis were convened to identify appropriate collection methods for each matrix and draft study designs to evaluate their performance. Protocols for the collection of surface sediments and aquatic biota are addressed in the current report (Part 1) whereas sample collection for stormwater flows will be addressed in a future report (Part 2).

APPROACH

A two-day workshop was held at the Southern California Coastal Water Research Project headquarters in Costa Mesa, California March 8-9, 2023, to convene an expert working group to develop standardized protocols for microplastics sample collection. Fifteen technical experts in microplastics sampling and analysis were invited to the working group. Technical experts included representatives from academia, government, wastewater management, industry, and non-governmental organizations. The workshop began with a series of presentations outlining the workshop process and goals. This was followed by a series of technical presentations for each matrix on the current state of the science for sample collection. Experts were then divided into breakout groups by matrix and tasked with selecting collection methods ready for evaluation, creating a prioritized list of key variables influencing sample collection methods, and drafting a study design for evaluating methods. Each working group presented their findings and proposed study design to the entire working group. To reach consensus, working group members voted on the proposed studies to identify which studies and matrices were the highest priority for experimental evaluation for the development of standardized protocols.

Following the workshop, sub-working groups were formed for each matrix to further develop proposed collection method evaluation studies and draft standard operating procedure (SOP) documents. This included informal literature searches to inform study designs and to determine if similar experiments had already been conducted. Sub-working groups met virtually over the next six months. A workplan was then drafted to outline a path forward for selecting and/or evaluating methods and drafting SOP documents.

RESULTS & DISCUSSION

Surface Sediment

Expert evaluation during the workshop identified sampling depth as having the greatest effect on the overall results when sampling sediment for microplastics analysis. Sampling depth is an

important factor to consider as many previous studies have found that concentrations generally decreased with sampling depth. This relationship has been observed in the ocean (Martin et al. 2017; Kukkola et al. 2022), bays (Zheng et al. 2020), estuaries (Fan et al. 2019), and other aquatic habitats (Yu et al. 2023). The relationship between sampling depth and microplastic concentration can be influenced by habitat, sediment deposition rates, as well as a variety of other factors (Yuan et al. 2023), but the majority of studies consistently report the highest concentrations in the top 2.5-5 cm of sediment (Martin et al. 2017; Fan et al. 2019; Zheng et al. 2020; Kukkola et al. 2022; Yu et al. 2023; Yuan et al. 2023). Monitoring programs often target the top 5 cm of sediment for sampling for other contaminants with the rationale being that most benthic organisms dwell in this layer (USEPA 2020). This sampling depth is also the most reported amongst microplastics occurrence studies (Hidalgo-Ruz et al. 2012) and has been recommended for other microplastic monitoring frameworks including, the European Marine Strategy Framework Directive, the Oslo Paris Convention, and method standardization efforts for microplastics (JRC 2013; Gerdts 2019; Bäuerlein et al. 2023). Sub-working group members agreed that 5 cm was a reasonable and technically sound sampling depth for routine monitoring of microplastics given that this depth typically represents the greatest concentration of microplastics by count, the sediment layer in which most aquatic benthic organisms reside and is consistent with previous recommendations and methods being employed around the world. This will ensure that microplastics are consistently detectable, informative of potential exposures and impacts to aquatic organisms, and that data are comparable to other microplastic monitoring programs.

In addition to sampling depth, experts identified the sampling device as another key variable for sediment sample collection. The primary concern amongst experts was that some sampling devices would excessively disturb sediments and cause microplastic loss. For example, if a spade is used to collect sediment and brought upward through the water column, both sediment and microplastic particles may wash from the sides during this maneuver. This effect is demonstrated when comparing spiked microplastic recovery rates using a Van Veen, a traditional sediment corer, a freeze corer, and a shovel with two different types of sediment under 20 cm of water under flow conditions (i.e., medium quartz sand and fine gravel) (Adomat et al. 2022). Though the diversity of spiked microplastics was limited (i.e., spherical microplastics ≥1 mm of four different polymer types), low recovery was observed for fine and low-density microplastic particles for the shovel (40-64%). The shovel was deemed unreliable for sampling as a general loss of microplastics was observed depending on the flow velocity of the water and the angle at which the shovel was held as it passed through the water column. The freeze core demonstrated greater recoveries (57-146%). However, rapid cooling may damage microplastics and cause fragmentation. Though recoveries varied based on the polymer and sediment type, grab samples using the Van Veen ranged from 39-87%. The sediment corer demonstrated the highest performance recovering 73-85% of microplastics.

Though the sediment corer outperformed the other devices, sampling device selection is partially dependent on sampling conditions and accessibility (e.g., wadeable versus unwadeable), and there are conditions where a corer may not be practical to use. Sub-working group experts agreed with the conclusions of the authors of this study that grab samplers, and coring devices were suitable for microplastics sampling depending on conditions and accessibility. Specifically, experts agreed that a Van Veen sampler is acceptable for non-wadeable conditions whereas a coring device is preferred in wadeable conditions. These sampling devices are also commonly used across monitoring programs, allowing microplastics sampling to be easily integrated in future surveys (USEPA 2020).

Surface sediment SOPs for microplastics sample collection prescribe the use of a Van Veen sampler or a sediment corer in non-wadeable and wadable conditions, respectively, at a sampling depth of 5 cm. Experts agreed that these sampling devices recover microplastics from sediment at a reasonable rate and have the advantage of being commonly used to collect sediments for environmental monitoring. Sampling at a depth of 5 cm can also inform microplastic exposure in benthic aquatic organisms given that most organisms reside in this layer of the sediment. Surface sediment collection SOPs are included in this report in Appendix A.

Aquatic Biota

The collection of aquatic biota for microplastics analysis is very similar to other environmental contaminants, but experts agreed that the target taxa and tissues selected should be carefully considered early in the planning stages for monitoring. It is recognized that taxa and species selection may be partly driven by availability, feasibility, and logistics. However, target species and tissue selection should be primarily driven by specific questions and objectives defined early on in the monitoring planning process (de Jourdan et al. 2024). For instance, if a monitoring program seeks to estimate human exposure to microplastics through fish consumption, it would be reasonable to target the edible tissues (e.g., fillet) of sportfish for analysis. Secondly, taxa selection should also consider differences in the amounts and types of microplastics that may be accumulated in an organism or tissue based on the physiological, biological, and ecological traits of that species (Franzellitti et al. 2019; Capparelli et al. 2022). Feeding behaviors directly influence microplastic exposure and accumulation. Organisms may only ingest microplastics that are smaller than their maximum gape size in at least two dimensions (Koelmans et al. 2020). Bivalves may recognize and reject some types of microplastics based on their size and morphology, and other species have been known to selectively ingest microplastics and avoid others based on specific particle characteristics (Ward et al. 2019, Horie et al. 2024). Pairing aquatic biota sampling with other relevant matrices (e.g., sediment, water) is recommended as it will allow researchers to determine how representative

the selected species are of the types and concentrations of microplastics in the surrounding environment (de Jourdan et al. 2024).

Experts provided guidance regarding sample size, minimum biomass, and pooling individual organisms for microplastics extraction and analysis. Sample sizes for any sample type should provide sufficient statistical power to address monitoring questions and objectives. Previous guidelines for fish and bivalves have recommended at least 50 individual organisms for each experimental permutation (e.g., species, feeding type, habitat, etc.) (JRC 2013; ICES 2015; Hermsen et al. 2018). Historical data, including data from peer-reviewed literature, may be used to predict microplastic concentrations and variability levels to conduct power analyses (de Jourdan et al. 2024). Similar datasets may be obtained through limited pilot studies prior to large-scale monitoring campaigns. Pooling organisms or tissues into a single sample may be necessary or desirable to achieve detectable amounts of microplastics or obtain more integrative samples. 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).

Aquatic biota SOPs for the collection of samples for microplastics analysis provide specific methods for the collection of bivalves (i.e., mussels and oysters) and fish, recognizing that these taxa are commonly targeted for contaminant monitoring, including microplastics and include procedures for mitigating and characterizing background contamination during dissection for the isolation of tissues. Methods may be adapted for the collection of other taxa that may be of interest for microplastics monitoring. Guidance on selecting appropriate sample sizes and tissue masses is also provided within the protocol. Biota collection SOPs are included in this report in Appendix B.

Considerations for Surface Sediment and Aquatic Biota

Experts agreed that mitigating, tracking, and characterizing background contamination is an essential part of microplastics research, including collection (Brander et al. 2020). Both sediment and aquatic biota collection protocols include detailed procedures for preventing background contamination whenever feasible. Requirements include, but are not limited to, the use of glass or metal instead of plastic with some specific exceptions (e.g., lab-grade tubing, nitrile gloves), equipment cleaning procedures using microplastics-free water, wearing clothing and cotton lab coats during sampling, and keeping all equipment covered with aluminum foil when not in use. A field blank (i.e., an aliquot of microplastic-free water in a sample container) is deployed and exposed to ambient conditions during sediment sampling to track background contamination. A similar sample, referred to as a dissection blank, is deployed if aquatic

organisms are dissected for the collection of internal tissues. The field blank and dissection blank are processed and analyzed to determine background contamination that occurred during sampling. Trip blanks are also employed to characterize contamination during transport. Field, dissection, and trip blanks may be used to identify sources of contamination if particles are fully characterized (i.e., particles are analyzed to determine size, morphology, color, and material type). The quantification and characterization of background contamination are essential to ensure reliability and reproducibility of results and establish analytical limits of detection and quantification.

Microplastic concentrations within each sample must be sufficiently greater than background contamination to reliably detect and quantify the number of microplastic particles present in the sample (Lao & Wong 2023). Detection limits for microplastics cannot be determined in the same way as that for soluble chemicals due to their discrete distributions (i.e., while measuring 1.4 ng/g of a chemical is possible, measuring 1.4 particles/g is not, only integer quantities such as 1 particle/g). Reducing background contamination will improve detection and quantification limits. However, it is also important to collect enough material (i.e., sediment or tissue) to ensure that microplastics can be detected and quantified. Specific procedures for calculating the minimum detectable amount (MDA) for microplastics are not within the scope of sample collection but are described in detail by Lao & Wong (2023) and in the drinking water SOPs for drinking water (SWRCB 2022a, 2022b). Sample collection procedures (i.e., the amount of material collected) will determine the likelihood that enough microplastics are collected and extracted for quantification (i.e., the number of microplastics in the sample collected are greater than the MDA). To aid in the planning process and help scientists estimate reasonable amounts of material to collect per sample, expert working group members developed a supplementary MDA and Sampling Unit Calculator in the form of a Microsoft Excel file that may be used for any matrix.

Finally, experts agreed that despite the standardization of collection methods, each monitoring study will be unique (e.g., objectives, sampling design, habitat, etc.). Therefore, there is a need to collect and report basic field data with the associated microplastics data so that results can be properly contextualized. Some required field data apply to all matrices (e.g., location, date, time, weather conditions, the sampling gear used, etc.), but there are also matrix-specific metadata. For sediment, this includes reporting the presence or absence of water and its condition (e.g., turbidity, color), and the number of sampling attempts (Appendix A, Table 3). Specific data to be reported with aquatic biota samples include the species name, habitat description, morphometric data (e.g., mass, length), and the total mass of the tissue or organisms to be analyzed for microplastics (Appendix B, Table 3). Additional data may be collected if deemed necessary or desirable as the data outlined in these protocols represents minimum requirements.

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APPENDIX A: STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF SURFACE SEDIMENT SAMPLES FOR MICROPLASTICS ANALYSIS

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. This method summarizes current best practices at the time of publication and more detailed guidance (e.g., sampling volumes, sampling devices, and approach) may continue to evolve through further study. This sampling protocol is intended to provide guidance for research purposes as well as the development of microplastic monitoring programs.

1.2 Sample processing and analysis methods are not within the scope of this method. Procedures for the extraction and measurement of microplastic particles in sediment have been tested and described in other related work (Langknecht et al. 2023, Thornton-Hampton et al. 2023).

1.3 Microplastics are ubiquitous environmental contaminants. It is impossible to eliminate background contamination from airborne particles, plastic or not plastic. This method provides recommendations for mitigating and characterizing background contamination during sample collection.

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

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

2.0 Summary of Method

This method 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 grab sampler (e.g., Van Veen sampler). Methods are consistent with commonly used sediment sampling methods, acknowledging that sampling may occur by leveraging existing monitoring campaigns for other contaminants. 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 2013; Gerdts 2019; U.S. EPA 2020). A minimum particle size of 50 µm for microplastics analysis is recommended as analytical methods have not yet been evaluated below this size range (State Water Resources Control Board 2022a, 2022b; Thornton Hampton et al. 2023).

3.0 Definitions

Field blank (FB) – An aliquot of MAG water (Microplastics Analysis Grade, see definition below) that is placed in a clean container in the laboratory before going into the field using contamination control procedures as described in Section 4. The FB is treated as a sample in all respects, including shipment to the sampling site, exposure to sampling site conditions, running through the sampling procedure, storage, preservation, and all analytical procedures. The volume of the FB must be similar to that of actual samples collected and processed by this method. A wetted filter placed in a clean glass petri dish may also be used. If using a wetted filter, the pore size should be less than the lower particle size limit of the study. The purpose of the FB is to determine if method analytes or other interferences are introduced into the samples during shipment and collection. At least one FB must be collected for each sampling event (see definition below). FBs differ from Trip Blanks (see definition below) in that the FB evaluates contamination during both shipment and collection, while the Trip Blank only accounts for contamination during shipment. Given that the FB is analyzed alongside field collected samples, the FB may also serve as the Laboratory Reagent Blank (LRB). If this approach is used, it is recommended that a subset of LRBs is analyzed separately to identify potential sources of contamination. For more details regarding the LRB, please see “Standard Operating Procedures for Extraction and Measurement by Infrared Spectroscopy of Microplastic Particles in Drinking Water” and “Standard Operating Procedures for Extraction and Measurement by Raman Spectroscopy of Microplastic Particles in Drinking Water” which

may be applied to other matrices as well (State Water Resources Control Board, 2022a, 2022b; Lao and Wong, 2023). If the FB plastic particle count is greater than the Minimum Reporting Level (see definition below), all associated field collected samples (i.e., collected during the same sampling event) must be flagged during data reporting.

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 (megaohm-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. For more details 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" which may be applied to other matrices as well (State Water Resources Control Board, 2022a, 2022b).

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

Trip Blank – A sample of MAG water of a similar volume as test samples, taken from the laboratory to the sampling site and returned without having been exposed to sampling procedures and the environment outside of the lab. The trip blank is to assess contamination

[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.

introduced during shipping and storage only and must be present for each set of field samples from a sampling event.

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 should 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 4.2.2.3 to 4.2.2.7.

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

4.2.2.9.1 A trip blank must be evaluated for shedding prior to the sampling event if different sampling containers from those listed in Section 6 are used. If total plastic particle counts are greater than the MRL, the sampling container may not be used. A trip blank is only analyzed if microplastic particle counts in the FB exceed the MRL and contamination is suspected to have occurred during transport.

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

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

4.2.4 Minimize the use of synthetic textiles in the field.

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

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

4.2.5 Clean all equipment thoroughly before use.

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

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

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

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

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

5.0 Safety

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

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

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

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

6.0 Sample Size and Material Amount Recommendations

While it is generally recommended that at least 100 g of sediment are collected for microplastic analysis, the amount of sediment collected should be enough to ensure that the amount of microplastics in the sample are at least above the Method Reporting Limit (calculated using the Minimum Detectable Amount (MDA) based on blank levels; see Lao and Wong, 2023). The amount of sediment collected will depend on the levels of microplastic pollution in the sampling regions and the procedural contamination. The 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.

7.0 Equipment and Supplies

7.1 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.

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

7.1.2 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.

7.1.3 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.

Table 1. Equipment list for surface sediment collection.

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 low-density polyethylene)	Avantor 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-ashed heavy-duty aluminum foil	-
Laboratory labeling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or low-density polyethylene)	Avantor no. 16651-904
Non-wadeable conditions: Van Veen grab sampler OR *Wadeable* conditions, no water: 5 cm diameter x 5 cm height metal cylinder OR *Wadeable* conditions, with water: Hand coring device with coring device cap	Wildco 1728-G30 grab dredge, 15 cm x 15 cm 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 12.2 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	-

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

Table 2. Equipment and supplies for the construction of a metal coring device.

Item	Suggested Materials
Core barrel	45 cm long, ~7.5 cm diameter metal or acrylic tube
Pipe cap with stainless steel tightening clamp	3 in. polyvinyl chlroide 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	-

7.2.1 If the pipe is longer than 45 cm, trim to appropriate length. Forty-five centimeters is a recommendation, but a few inches longer or shorter is acceptable if more ergonomically comfortable.

7.2.2 If it is anticipated that sediment may be difficult to penetrate using an acrylic tube, a file, Dremel tool or other preferred device, may be used to cut a bevel at one end of the tube, which will become the bottom (Figure 1). This is suggested only if deemed necessary as this process may introduce microplastic contamination.

7.2.3 Mark the depth guide for inserting the core into the sediment. Measure 5 cm up from the bottom of the core (i.e., beveled end or end designated for initial penetration into the sediment) and wrap weatherproof tape or indelible marker around the core to mark it.

7.2.4 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 large enough to fit the rubber stopper securely. Insert the rubber stopper.

7.2.5 If a handle for the coring device is desired, affix the riser clamp approximately 30-40 cm 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).

7.2.6 Ensure rubber cap fits the coring device.

7.2.7 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.

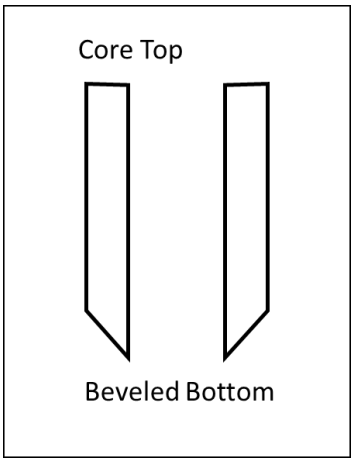

Figure 1. Diagram of beveled core bottom for better sediment penetration. The diagram shows two symmetrical vertical rectangles, positioned side by side with sharp diagonal cuts on the bottom corners.

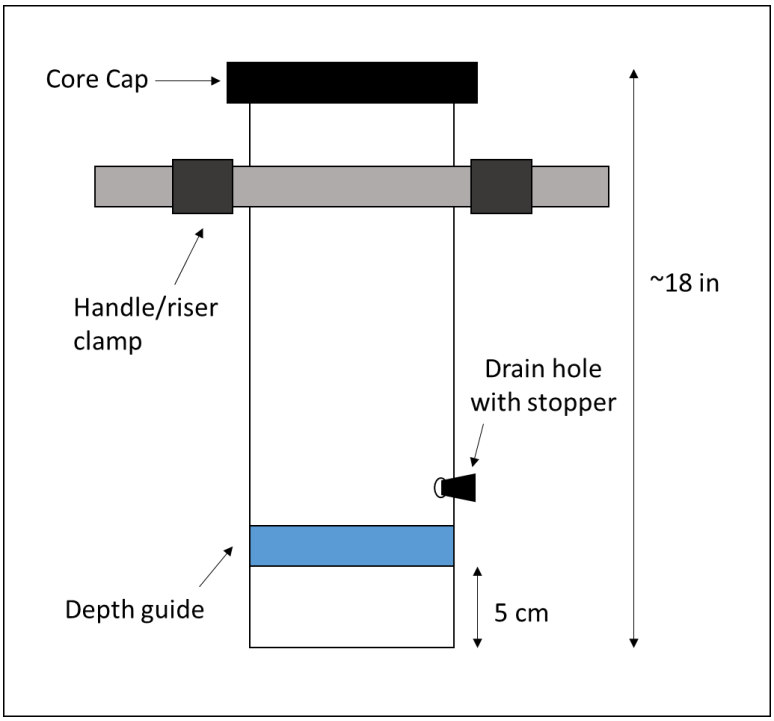

Figure 2. Basic diagram of coring device. The central section of the device is represented by a vertical white rectangle. At the top, a horizontal black rectangle represents the core cap. Directly below the top a horizontal grey rectangle extends beyond the width of the central section to represent the handle and riser clamps. A small circle near the bottom with a black isosceles trapezoid represents the drain hole with a stopper. Five centimeters from the bottom of the central section is a blue horizontal rectangle representing the depth guide. The total height of the coring device is approximately 18 inches.

8.0 Reagents and Standards

8.1 MAG water is required throughout the cleaning and sampling process to ensure that equipment and sampling jars are free of particle contamination. The MAG water is to be collected and stored in a clean vessel (Section 4.2.5.1) and covered (Section 4.2.5.4) until use.

8.2 The FB should be created by the laboratory by adding 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.

9.0 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.

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

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

9.3 Contamination Control Verification – It must be verified that plastic equipment that comes into contact with samples does not shed particles. Rinse each piece of equipment three times with MAG water, collecting the rinsate in a clean sampling jar (see Section 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 prior to each sampling campaign or biannually, whichever is less frequent (Section 4.2.2.9).

10.0 Procedure

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

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

10.1.1 Rinse the Van Veen grab sampler with site water three times, followed by MAG water three times.

10.1.2 Deploy the Van Veen to collect sediment.

10.1.3 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.

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

10.1.5 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.

10.1.6 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 a high-quality cotton cloth or paper towel.

10.1.7 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.

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

10.2 Sampling via hand coring device in wadeable conditions.

10.2.1 Identify an undisturbed ~1.5 x 1.5-meter area without vegetation, taking care not to step in the sampling area.

10.2.2 Rinse the coring device with site water three times, followed by MAG water three times.

10.2.3 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.

10.2.4 Push the coring device into the sediment to a depth of at least 5 cm. The coring device may be slightly twisted while pushing if sediments are difficult to penetrate. This may be the top of the coring device if using a 5 cm metal cylinder or to the depth guide marked on the outside of the core barrel.

10.2.4.1 If water is over the sampling area, cap the top of the coring device once it has been inserted into the sediment. This will help to prevent sediment from being disturbed or falling out of the bottom of the coring device when extracted.

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

10.2.6 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.

10.2.7 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 11.3), 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.

> 10.2.7.1 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. Discard excess sediment well outside of the previously defined ~1.5 x 1.5-meter sampling area.
>
> 10.2.7.2 If excess water is trapped inside the coring device, it may be drained outside of the previously defined ~1.5 x1.5-meter sampling area 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.

10.2.8 Repeat steps described in Sections 10.2.4 to 10.2.7 until the desired amount of sediment or replicate samples have 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 ~15 to 45 cm apart within the sampling area where the sediment has not been previously disturbed.

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

11.0 Sampling Acceptability Criteria

11.1 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.

11.2 Acceptability for grab samples collected using a Van Veen under non-wadeable conditions is based upon two characteristics: sample condition and depth of penetration. If it is not possible to collect an undisturbed sample after multiple attempts, details regarding the degree of disturbance should be recorded.

11.2.1 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.

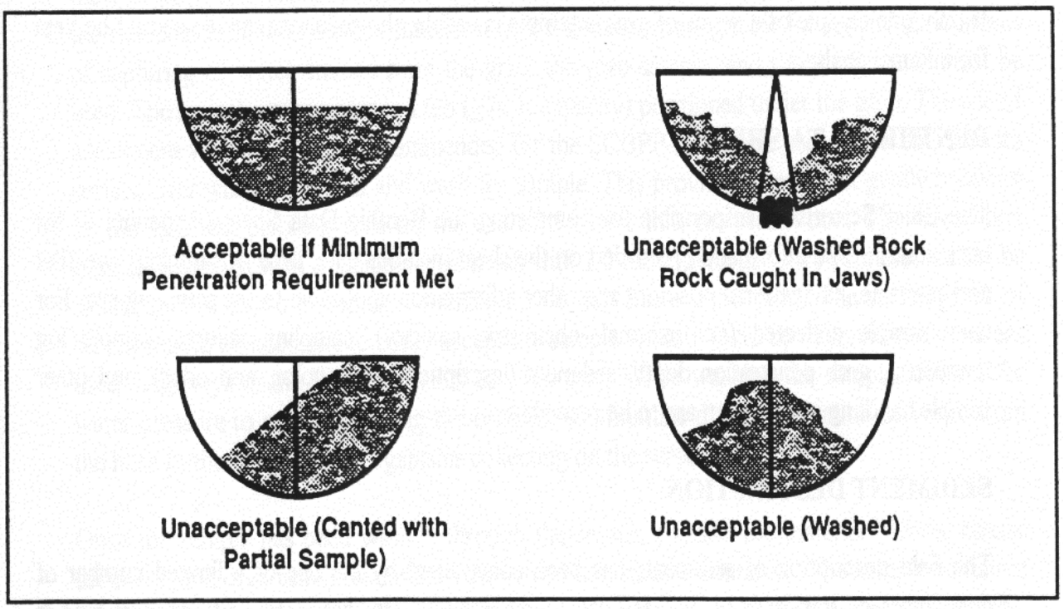

Figure 3. Examples of acceptable and unacceptable grab sample conditions. Top left: "Acceptable If Minimum Penetration Requirement Met" shows a sample with evenly distributed material across the semicircular container. Top right: "Unacceptable (Washed Rock Rock Caught In Jaws)" depicts uneven material with rock caught in the jaws, leaving a gap in the center. Bottom left: "Unacceptable (Canted with Partial Sample)" shows an uneven, slanted sample occupying only part of the container. Bottom right: "Unacceptable (Washed)" displays a washed-out sample with a central peak and insufficient material.

11.2.2 Sample condition may be recorded by taking a photo.

11.2.3 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.

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

11.3.1 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.

11.3.2 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.

12.0 Sample Preservation and Storage

12.1 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-ashed, heavy-duty aluminum foil. It is not recommended that samples be frozen, but, if necessary, one freeze thaw cycle is acceptable (-20°C).

12.2 Glass containers with non-plastic lid liners (PTFE is acceptable if not also sampling for PFAS), pre-cleaned as with other apparatuses (Section 4.2.5) in this method, must be used to collect and store samples to minimize microplastic contamination from the container when feasible. If there is concern regarding breakage during storage, travel, or shipment, other containers may be used as long as they are evaluated for shedding (Section 8.3). Containers

shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts or other packing materials that may easily fragment if possible; if not, then ensure that containers are sealed prior to shipment and the outsides are rinsed thoroughly before the sample is opened to prevent contamination. Shipping samples on ice (< 6 °C) is preferred, but samples may be shipped at room temperature.

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 FBs.

12.4 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.

13.0 Field Data Reporting Requirements

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

Table 3. Field data reporting requirements for surface sediments.

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 wadeable or non-wadeable?
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 fabric type (i.e., material, color) of apparel worn
Sampling Attempts	Number of sampling attempts made before acceptable sample obtained (see Section 11)
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

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 landfill.

15.0 References

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APPENDIX B: STANDARD OPERATING PROCEDURES FOR THE COLLECTION OF AQUATIC BIOTA SAMPLES FOR MICROPLASTICS ANALYSIS

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. This method summarizes current best practices at the time of publication and more detailed guidance (e.g., tissue amounts, sampling devices, and approach) may continue to evolve through further study. This sampling protocol is intended to provide guidance for research purposes as well as the development of microplastic monitoring programs.

1.2 Sample processing and analysis methods are not within the scope of this method. Procedures for the extraction and measurement of microplastic particles in biota have been tested and described in other related work (Thornton-Hampton et al. 2023).

1.3 Microplastics are ubiquitous environmental contaminants. It is impossible to eliminate background contamination from airborne particles, plastic or not plastic. 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 Program and/or 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 or monitoring program. 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. A minimum particle size of 50 µm for microplastics analysis is recommended as analytical methods have not yet been evaluated below this size range (State Water Resources Control Board 2022a, 2022b; Thornton Hampton et al. 2023).

3.0 Definitions

Dissection blank (DB) – An aliquot of MAG water (Microplastics Analysis Grade, see definition below) that is placed in a clean container in the laboratory before dissections begin using contamination control procedures as described in Section 4. The DB is then treated as a sample in all respects, including running through the sampling procedure, storage, preservation, and all analytical procedures. If dissections are to be conducted in the field, this also includes shipment to the sampling site and exposure to sampling site conditions. The volume of the DB must be similar to that of actual samples collected and processed by this method. A wetted filter placed in a clean glass petri dish may also be used. If using a wetted filter, the pore size should be less than the lower particle size limit of the study. The purpose of the 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. At least one DB must be collected for each dissection event (see definition below). DBs differ from Trip Blanks (see definition below) 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” which may be applied to other matrices as well (State

Water Resources Control Board, 2022a, 2022b; Lao and Wong, 2023). If the DB plastic particle count is greater than the Minimum Reporting Level (see definition below), all associated field collected samples (i.e., collected during the same sampling event) must be flagged during data reporting.

Dissection event – Dissection that occurs within a 24-hour period in the same location under consistent conditions with the same laboratory or field crew collecting the same set of tissues from the same species.

Microplastics – Solid[3] polymeric materials[4] 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 8). 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 (megaohm-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. For more details, 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" which may be applied to other matrices as well (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

[3] '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.

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

procedures and the environment outside of the lab. The trip blank is to assess contamination introduced during shipping and storage only and must be present for each set of field samples from a sampling event..

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 should 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 4.2.2.6.

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, horsehair brush).

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

4.2.2.4 Typical laboratory-grade solvent squeeze bottles (e.g., Teflon or polyethylene) are also suitable to dispense MAG water for the rinsing of sampling devices, sampling jars, and other equipment as long as they are used similarly for QA/QC samples (e.g., 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). Glass jars may also be used, but it should be noted that potassium hydroxide, which is used to digest tissues, may etch glass.

4.2.2.7 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 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 biannually using the procedures noted in Section 11 – Quality Control.

4.2.2.8.1 A trip blank must be evaluated for shedding (Section 4.2) prior to the sampling event if different sampling containers from those listed in Sections 8 and 9 are used. If total plastic particle counts are greater than the MRL, the sampling container may not be used. For biota, a trip blank is only analyzed if dissections occur in the field, microplastic particle counts in the DB exceed the MRL, and contamination is suspected to have occurred during transport.

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 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.

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

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

4.2.3.5 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).

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. Remove or replace materials as necessary. For items that cannot be removed or replaced that may contribute to contamination, it is recommended to photograph and document their properties.

4.2.4.3 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.

4.2.4.4 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.

5.0 Safety

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

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

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

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

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 two dimensions

(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 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).

It is recommended that organism selection criteria (e.g., minimum mass/body length, life stage, condition) are determined during planning stages, well in advance of sample collection.

If more granular data are desired, individual organisms should be analyzed as opposed to composite samples (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 Minimum Detectable Amount (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 (see Lao & Wong 2023 for details).

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.

Table 1. Equipment and supplies for bivalve sample collection.

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 low-density polyethylene)	Avantor 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 labeling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or low-density polyethylene)	Avantor 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 labeling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or low-density polyethylene)	Avantor no. 16651-904
Measuring tape or ruler	-
Ethanol	-

Item	Suggested Materials
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 (~5 gallon)	-
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 rubber gloves (ideally cut resistant)	Grainger (Item 56FK94)
Shucking knife	-
Calipers	Avantor 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 hard substrates.

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.

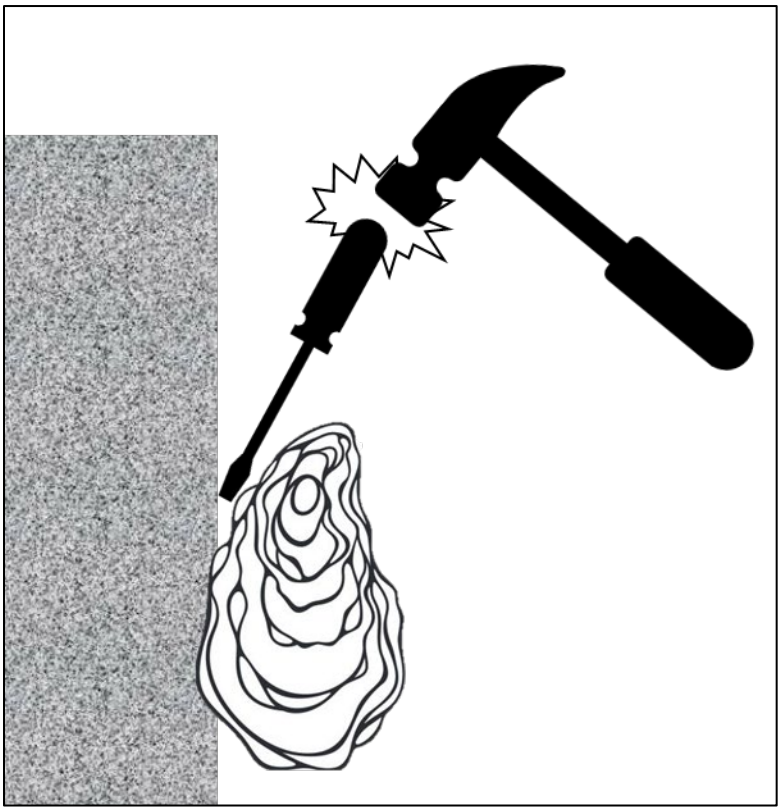

Figure 1. Removal of bivalve from hardscape using mallet and chisel. A hammer taps the back of screwdriver. The tip of the screwdriver is wedged between a bivalve and a rectangle representing a hardscape.

8.3.2.3 Upon collection, rinse each bivalve in site 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.

8.4.5 Upon collection, rinse each clam in site water to remove excess debris and wrap each individual clam 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.

8.5 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 (i.e., remove the jar or petri dish lid). 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 rubber 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 and associated fluids on the foil 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).

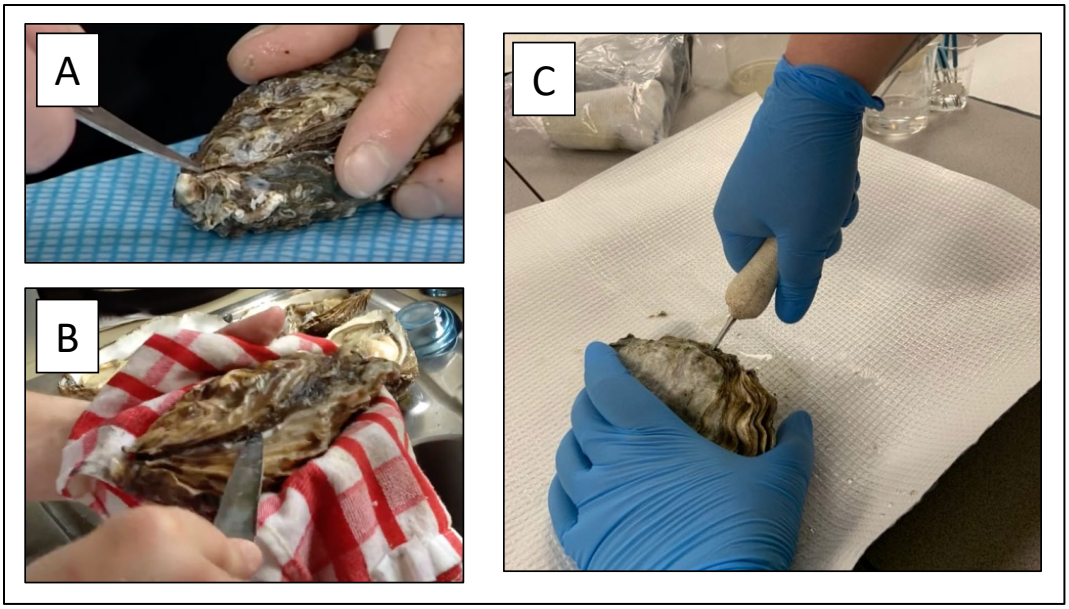

Figure 2. (A) Example of piercing from hinge. (B) Example of piercing from un-frilled side. (C) Example of how deep the shucking knife should be inserted into the bivalve for shucking. Note that these images are to demonstrate shucking techniques and that bivalves should be opened over a pre-ashed piece of heavy-duty foil while wearing heavy-duty rubber gloves for protection.

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.

Table 2. Equipment and supplies for fish sample collection.

Item	Suggested Materials
Fish Sample Collection	
Nitrile gloves	Kimtech™ Purple Nitrile™ Exam Gloves (Product code #55083)
Pre-ashed heavy-duty aluminum foil	-
Laboratory labeling tape	Fisher Catalog no. 15901A
Squeeze bottle (Teflon, polypropylene, or low-density polyethylene)	Avantor 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	Avantor Catalog no. 36934-152
Kim Wipes	-
Balance	-
Polypropylene sample jars	Avantor Catalog no. 30617-164
Dissection	
Pre-ashed heavy-duty aluminum foil	-
Calipers	Avantor Catalog no. 36934-152
High quality cotton cloth or paper towels	-
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; Avantor, (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).

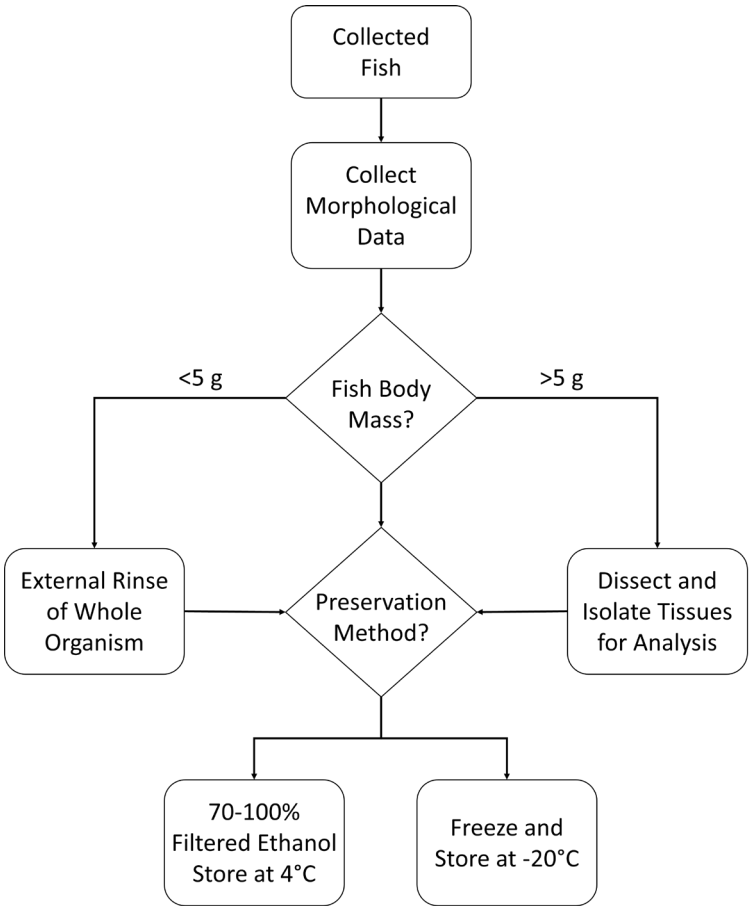

Figure 3. Decision tree for sampling and preserving fish on the basis of body size. Fish and their morphological data are collected. If the total body mass of the fish is less than five grams, the outside of the organisms is rinsed with microplastic analysis grade water. If the total body mass of the fish is greater than five grams, it is dissected and tissues targeted for analysis are isolated. Whole fish or isolated tissues may be stored in 70-100% filtered ethanol at 4°C or frozen at -20°C.

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 cotton cloth or paper towel 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 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 (i.e., remove the jar or petri dish lid). 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 cotton cloth or paper towel 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 4.2.3.4) 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 MAG water to a clean (Section 4.2.4.1) sampling jar. If a wetted filter is used, a filter with a pore size no larger than the minimum target particle size is placed in a clean petri dish. Upon dissection, the filter is wetted with MAG water to ensure particles adhere to the filter. Additional MAG water may be added periodically to ensure that the filter does not dry out. One DB should be prepared for every set of samples dissected at the 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 (section 3) must be included with each set of samples dissected at the same time and analyzed to assess contamination during dissection. 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 (Section 3) must be evaluated before the sampling event if a different type of sampling container is used from those listed in Sections 8 and 9. Trip blanks do

not need to be analyzed unless the DB shows evidence of contamination (i.e., microplastic levels in the DB are greater than the MRL) and contamination is suspected to have occurred during travel or shipment. The Trip blank may be analyzed to determine if contamination occurred during shipping or travel.

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, 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 prior to each sampling campaign or biannually, whichever is less frequent (Section 4.2.2.9).

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 organisms 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 DBs.

12.4 If there is concern regarding breakage during storage, travel, or shipment, other containers may be used as long as they are evaluated for shedding (Section 8.3). Containers shall be securely packaged to avoid breakage during shipment. Avoid the use of plastic packing peanuts or other packing materials that may easily fragment if possible; if not, then ensure that containers are sealed prior to shipment and the outsides are rinsed thoroughly before the

sample is opened to prevent contamination. Samples must be freeze dried or shipped on ice (< 6 °C).

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.

Table 3. Field reporting requirements for biota sampling.

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 fabric type (i.e., color and material type) of apparel worn.
Habitat Type	Description or photos 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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