2023 Southern California Bight Regional Monitoring Survey

Bight ‘23 Trash and Microplastics Workplan

3 June 2024

Prepared by the Bight ‘23 Trash and Microplastics Planning Committee

Table of Contents

Trash and Microplastics Planning Committee Membership

Alvine Mehinto	Southern California Coastal Water Research Project Authority
Ami Latker	City of San Diego, Public Utilities Department
Andrea Bonisoli-Alquati	California State Polytechnic University, Pomona
Andrew Gray	University of California Riverside
Susanne Brander	Oregon State University
Charles Wong	Southern California Coastal Water Research Project Authority
Chiharu Mori	National Oceanic and Atmospheric Administration
Christy Kehoe	National Oceanic and Atmospheric Administration
Danny Tang	Orange County Sanitation District
Emily Parker	Heal the Bay
Erika Holland	California State University Long Beach
Hannah Hapich	University of California Riverside
Jay Anderson	Photothermal
Jennifer Smolenski	City of Los Angeles Bureau of Sanitation
Jian Peng	Orange County Public Works
Jo Ann Weber	San Diego County Watershed Protection Program
Jojo Loan	Los Angeles County Sanitation Districts
Jon Ball	City of Los Angeles Bureau of Sanitation
Joshua Westfall	Los Angeles County Sanitation Districts
Kaitlyn Kalua	Ocean Protection Council
Karen McLaughlin	Southern California Coastal Water Research Project Authority
Karin Wisenbaker	Aquatic Bioassay and Consulting Laboratories
Katie Kelly	Orange County Public Works
Kelly Hahs	City of Ventura
Ken Sakamoto	Orange County Sanitation District
Ken Schiff	Southern California Coastal Water Research Project Authority
Lauren Valentino	City of San Diego, Public Utilities Department
Leah Thornton Hampton	Southern California Coastal Water Research Project Authority
Mark Baker	Physis Labs
Michael Mori	Orange County Public Works
Rae McNeish	California State University Bakersfield
Ryan Kempster	City of San Diego, Public Utilities Department
Saffron Hullinger	Aquatic Bioassay and Consulting Laboratories
Samantha Leigh	California State University Dominguez Hills
Samiksha Singh	University of California Riverside
Seth Jones	Marine Taxonomic Services
Shelly Walther	Los Angeles County Sanitation Districts
Stacee Karnya	City of Los Angeles Bureau of Sanitation
Susan Cheng	City of Los Angeles Bureau of Sanitation
Terra Petry	Los Angeles County Sanitation Districts
Ting Yang	Photothermal
Victoria Kalkirtz	City of San Diego, Public Utilities Department
Violet Renick	City of San Diego, Public Utilities Department
Win Cowger	Moore Institute for Plastic Pollution Research
Zoë Scott	City of San Diego, Public Utilities Department

List of Figures

List of Tables

Introduction

The pollution of oceans and watersheds by anthropogenic litter has been recognized as a serious global environmental concern (Amon et al., 2020). On land and in freshwater habitats, this litter is typically referred to as trash, and as marine debris in ocean habitats. Trash and marine debris affect aesthetics as well as habitat quality and aquatic life. Leggett et al., (2014) found that marine debris in southern California significantly influences the decision of the public to go to beaches, costing Orange County residents alone an estimated $148 million per year just to travel to cleaner beaches. In marine environments, debris presents entanglement and ingestion dangers for marine organisms (Boerger et al., 2010; Goldstein and Goodwin 2013; Lusher et al., 2013; Anastasopoulou et al., 2013; Bond et al., 2013; Di Beneditto and Ramos 2014; Gall and Thompson 2015). Furthermore, plastics in the environment can transport other contaminants, creating a bioaccumulation pathway by which aquatic organisms take up contaminants when they consume plastic (Rios et al., 2007; Farrington and Takada 2014).

Because trash and marine debris have the potential to adversely impact freshwater and marine beneficial uses, California state and local agencies have proposed and implemented trash mitigation strategies for California waterways, including recycling programs, plastic bag bans, and other regulations and legislation. In 2011, California Assembly Bill 341 established a goal of reaching 75% recycling statewide by 2020 through source reduction, recycling, and composting (CalRecycle 2015). State and federal regulators also have established total maximum daily loads (TMDLs) for the Los Angeles River and Santa Monica Bay watersheds, specifying that their rivers contain zero trash pieces greater than 5 mm in diameter (CRWQCBLAR 2007, 2010, 2015, 2019). The State Water Board adopted similar statewide regulations as part of its Water Quality Control Plan (SWRCB 2015). California Senate Bill 270 (SB 270) in 2014 issued a statewide ban on single-use plastic bags and California voters approved Proposition 67, confirming their support of the ban that was then implemented state-wide in 2016.

The Southern California Bight (SCB) 2013 Regional Monitoring Program (Bight '13) included the first, coordinated regional assessment of trash and marine debris in the Southern California Bight. This study found that trash was pervasive in both streams and offshore, observing trash in three quarters of SCB wadable streams and one third of the seafloor. This study also found offshore marine debris has been increasing from 1994 to 2013. Trends were not assessed for watershed trash because 2013 was the first time a coordinated watershed trash assessment was conducted. The study was repeated in The Southern California Bight 2018 Regional Monitoring Program (Bight '18). This study identified similar extent and magnitude of Trash as the 2013 survey, but also found indications that management actions may have decreased trash and plastic in SCB watersheds. The Santa Monica Bay watershed saw a significant decrease in both trash and plastic abundance, perhaps due to the trash TMDL in the watershed years prior. In addition, abundance of plastic bags significantly decreased between 2013 and 2018, perhaps due to the statewide bag ban implemented in 2016.

While previous Bight programs have focused on large debris items, there has been increasing concern regarding the potential impacts of microplastics, plastic particles less than 5 mm in size. California's first piece of legislation that specifically applied to microplastics was passed in 2008, when strict regulations were placed on the manufacture, handling, and transport of pre-production plastic pellets. Then, in fall 2015, Assembly Bill 888 prohibited the sale of personal care products containing plastic microbeads—only a few months prior to enactment of a similar federal ban. Most recently, in 2018, the California Legislature adopted a pair of bills that require the State to begin building microplastics management strategies for both drinking water and California's coastal ocean and estuaries. Senate Bill 1422 requires the California State Water Resources Control Board to develop plans for measuring microplastic particles in drinking water by 2021. Senate Bill 1263 requires the California Ocean Protection Council to adopt and implement a statewide strategy for lessening the ecological risks of microplastics to coastal marine ecosystems.

To evaluate the effectiveness of management actions and establish linkages with regional factors which may result in changing amounts of trash and marine debris, continued monitoring of trash and marine debris is required. Long-term data on trash types, as well as trash extent and magnitude, can be used to target items for bans as well as track the effectiveness (or lack thereof) of specific management actions (including bans as well as trash mitigation strategies), identify hotspots, and generate enough statistical power to evaluate sources and pathways for trash pollution in receiving waters. In addition, there is a need to determine current environmental conditions regarding the extent and magnitude of microplastics in the Southern California Bight. The Bight '23 Trash and Microplastics Program was designed to meet these needs.

2023 Survey

The proposed Southern California Bight 2023 Regional Monitoring Program (Bight '23) is a continuation of the successful cooperative regional-scale monitoring in southern California. Bight '23 builds upon the previous successes and expands on the 2018 program by including new participants, answering additional questions, and measuring more parameters. Forty eight organizations, including international and volunteer organizations, have agreed to participate. The inclusion of multiple participants, many of them new to regional monitoring, provides several benefits. Cooperative interactions among many organizations with different perspectives and interests, including a combination of regulators and dischargers, ensure that an appropriate set of regional-scale questions will be addressed by the study.

The Bight '23 Program is organized into six technical components: 1) Sediment Quality (formerly Contaminant Impact Assessment/Coastal Ecology), 2) Microbiology, 3) Ocean Acidification, 4) Harmful Algal Blooms, 5) Estuaries and Wetlands, and 6) Trash and Microplastics. The Trash and Microplastics component focuses on 1) the types, amounts, and extent of trash in the ocean, in estuaries and wetlands, and in coastal rivers and streams and 2) the extent and magnitude of microplastics in the coastal environment. This will be the first time microplastics have been included as part of the Bight Program. This Workplan provides a summary of the trash and microplastics objectives and project design. Separate Workplans are also available for the other elements of Bight '23.

Trash

Study Objectives

The overall objective of the Bight '23 Trash Survey is to characterize the extent and magnitude of trash in the SCB watersheds and coastal habitats. Specifically, there are three questions of interest:

1. *What is the extent and amount of trash on the SCB seafloor, estuaries, and inland waterways?*
2. *What are the trends of trash types and amounts on the SCB seafloor, estuaries, and inland waterways?*
3. *Are there any factors that may be contributing to larger amounts of trash in estuaries and inland waterways?*

The first question seeks to understand the total amounts of trash found on the SCB seafloor, estuaries, and inland waterways. The second question intends to understand the relative amounts of different trash items amongst strata. Finally, the third question seeks to understand the relationship between the amounts and types of trash and selected explanatory factors such as land use.

Trash in Inland Waterways

Background and Objectives

Trash on land has recently become a focus of policy throughout the state of California. These policies include three main areas: 1) bans; 2) total maximum daily loads (TMDLs); and 3) the Statewide Trash Amendments. While these policies all involve reducing trash on land, they all work at different levels. Bans on specific items include the statewide ban on plastic bags (SB270), and local bans throughout the state on items such as polystyrene and cigarettes. Regional water quality control boards have passed TMDLs on many contaminants and specifically on trash for at least 15 water bodies. The most well-known TMDL for the Los Angeles River was one of the nation's first trash TMDLs and was established in 2001. The goal of 100% trash load reduction for this TMDL was set to be accomplished by September 2016. Many jurisdictions are attempting to accomplish this using full trash capture devices or alternative institutional controls such as street sweeping, education, etc. The Statewide Trash Amendments take the TMDLs to a larger scale, as jurisdictions throughout the state now must either install full trash capture devices (Track 1) or partial capture devices and institutional controls (Track 2). For those opting for Track 2, monitoring is required to ensure they are attaining results comparable to Track 1 areas.

Few studies have been done to examine trash in rivers and streams within urban settings. Much of the information on trash in these systems comes from Public Works Agencies and are estimated based on gross measurements, such as the weight and/or volume of the overall or categorical loads. In 2011-2013 the Southern California Stormwater Monitoring Coalition (SMC), as part of their larger Regional Stream Survey, incorporated a trash survey and sampled sites throughout southern California in a wide variety of habitats. These surveys were incorporated into the Bight 2013 and 2018 Regional Surveys, which included other participants, such as Orange County and San Diego Coastkeeper. The results of these studies demonstrated that trash is highly prevalent in streams, particularly in urban and agricultural areas. The extent and magnitude of trash in inland waterways was similar between the two surveys. However, there was evidence that management actions (i.e., plastic bag bans) may be having an impact on reducing the prevalence of some trash types (McLaughlin et al., 2022). While these studies provided a baseline for Southern California stream trash, more information is necessary to determine if trash numbers are going up, staying the same, or declining.

The goal of the Bight '23 Trash survey will be to determine the amounts and types of trash in the channels of wadable streams. Trash deposited in riverine and estuarine habits occurs through several primary processes, including but not limited to 1) land use-based sources, 2) incidental or wind-blown debris from adjacent areas, and 3) direct deposit of debris through littering and illegal dumping. Understanding the amounts and types of debris in riverine habits is a first step in making the connections between land-based sources and debris that is ultimately transported to the ocean.

The Bight '23 Trash survey in inland waterways complements the existing SMC effort to assess trash with a greater focus on the urban stratum. The objectives of this riverine habit study component include three main questions focusing not only the magnitude and extent of trash in rivers, but also on factors that may influence them (**Table 1**).

Table 1. Summary of management questions, study objectives, and intended data applications of the Bight '23 Regional Monitoring Trash survey in inland waterways.

Management Question	Study Objective	Application
What is the extent and amount of trash in inland waterways?	Assess the amount and spatial distribution of trash in rivers and streams	Determine the overall trash condition of rivers and streams
What are the trends of trash types and amounts in inland waterways?	Assess the relative quantities of different trash types in rivers and streams	Compare to Bight '13 and '18 surveys to determine if conditions are improving or declining
Are there any factors that may be contributing to larger amounts of trash in inland waterways?	Determine if trash abundance or specific types of trash are associated with selected factors	Identify factors leading to high trash abundance or high prevalence of specific trash types as possible targets for future management actions

Conceptual Approach

Trash assessments in inland waterways will include a qualitative visual assessment (Tier 1, California Trash Monitoring Playbook) and a quantitative tally assessment (Tier 4, California Trash Monitoring Playbook). In streams, a 30-meter stream reach is used to determine an overall visual score (i.e., low, moderate, high, or very high) and tally individual trash items. These assessments will be completed by leveraging existing monitoring efforts by the Southern California Stormwater Monitoring Coalition (SMC).

Target Population, Sample Frame Development, and Site Selection

Because this study component is being leveraged over resources in place through the SMC Regional Watershed Monitoring Program, the target population, sample frame, and site selection has been pre-determined by that workgroup. The target population for the 2023 SMC survey is wadable and Strahler second order or higher classification streams across the Southern California watersheds. The sample frame will include the major strata used in previous SMC surveys, which are as follows:

1. Strahler Order
2. Land Use
    1. Urban
    2. Agriculture
    3. Open
3. Watershed Jurisdiction (Hydrologic Unit Boundaries)
4. County Jurisdictional Boundaries
5. Regional Water Quality Control Board Jurisdiction Boundaries

Sample sites were selected using a probabilistic approach weighing by watershed, land use, and stream order. The sampling frame includes watershed units located from Ventura to San Diego and as far east as San Bernardino and

Riverside Counties. These watersheds equate to combinations of management units utilized by the Regional Water Quality Control Boards (RWQCB) or SMC member agencies. Altogether, these 15 watershed units are comprised of roughly 28,051 km^2. The streamlines used to define the sampling frame were derived from the National Hydrography Dataset (NHD Plus). Altogether, there are 9,492 stream miles of Strahler order 2 and greater in the sampling frame. Land use was defined as either urban, agriculture, or open based on Coastal Change Analysis Program remote imaging algorithms (National Oceanic and Atmospheric Administration 1995). CCAP defines 35 different land use classes that have been aggregated into the three categories for this study (i.e., open, agriculture, and urban). The dominant land use within a 500-m buffer was assigned to each stream reach.

Sampling and Analysis Methods

The approach required to conduct trash assessments in streams will utilize the California Trash Monitoring Methods and Assessments Playbook protocols (hereafter California Trash Monitoring Playbook) (Moore et al., 2020), specifically Tier 1 and Tier 4 methods. In addition to the qualitative analysis, individual debris items will be recorded according to specific item categories on the Stream Trash Item Tally Sheet. Steps 1-6 of each method are identical. All protocols and data sheets are adapted from **California Trash Monitoring Playbook** and can be found in **Appendix A**.

Proposed Deliverables and Timeline

Table 2. Proposed tasks and deliverables and timeline for Trash in Inland Waterways.

Task/Deliverables	Estimated Completion Date
Field Deployment	Summer 2023
Data Submission	Fall 2023
Data Analysis	Spring 2024
Draft Report	Fall 2024
Final Report	Winter 2024

Trash in Estuaries and Wetlands

Background and Objectives

Trash assessments in inland streams and on the seafloor have been ongoing since Bight '13 and Bight '94, respectively. Yet, trash has not been assessed in estuaries and wetlands as part of the Bight Regional Monitoring Program. These coastal habitats are of interest as they represent the interface between inland and marine environments in addition to the numerous ecosystem services that they provide. Trash assessments in estuaries and wetlands will provide critical baseline data for the extent and magnitude of trash in these habitats. Therefore, the goal of the inaugural Bight '23 Trash survey will be to determine the quantities and types of trash in coastal estuaries and wetlands.

Estuary and wetland trash assessments will leverage planned efforts by the Estuary Wetlands Bight '23 working group. Specifically, marsh plain vegetation surveys will be adapted to also include trash surveys in key habitat zones in the marsh platform and intertidal zone. Data will be collected in a similar manner to inland streams with some modification, including both a qualitative visual assessment (Tier 1, California Trash Monitoring Playbook) and a quantitative tally assessment (Tier 4, California Trash Monitoring Playbook).

The objectives of this estuaries and wetlands habit study component includes three main questions focusing not only the magnitude and extent of trash in estuaries and wetlands, but also on factors that may influence them (**Table 3**).

Table 3. Summary of management questions, study objectives, and intended data applications of the Bight '23 Regional Monitoring Trash survey in estuaries and wetlands.

Management Question	Study Objective	Application
What is the extent and amount of trash in estuaries and wetlands?	Assess the amount and spatial distribution of trash in estuaries and wetlands	Determine the overall trash condition of estuaries and wetlands
What is the extent and amount of specific trash types in estuaries and wetlands?	Assess the types of trash present in estuaries and wetlands	Determine which trash types are most prevalent in estuaries and wetlands
What factors contribute to larger amounts of trash and specific trash types in estuaries and wetlands?	Determine if total trash amounts or specific types of trash are associated with selected factors	Identify factors leading to high trash abundance or high prevalence of specific trash types as possible targets for future management actions

Conceptual Approach

Trash assessments in estuaries and wetlands will leverage efforts planned by the Estuary Wetlands Bight '23 Element, specifically the marsh plain vegetation transect surveys (California Estuary Marine Protected Area Monitoring Program, SOP 11). One-meter quadrats are distributed along transects (minimum length of 25m) across the marsh platform from the intertidal to the upland habitat. While field crews conduct vegetation surveys, they will also assess the occurrence of trash within the quadrats. Trash assessments will be conducted by adapting methods used in inland streams, including a qualitative visual assessment (Tier 1, California Trash Monitoring Playbook) and a quantitative tally assessment (Tier 4, California Trash Monitoring Playbook). In practice, field

crews may conduct vegetation and trash surveys simultaneously. However, estuary trash assessment methods are presented separately in this workplan. Estuaries and the stations within each estuary will be preselected by the Estuary Wetlands Bight '23 Element.

Sampling and Analysis Methods

The approach required to conduct trash assessments in estuaries and wetlands will adapt the California Trash Monitoring Playbook protocols used for inland streams, specifically Tier 1 and Tier 4 methods. In addition to the qualitative analysis, individual debris items will be recorded according to specific item categories on the **Estuary Trash Item Tally Sheet**. All protocols and data sheets are adapted from **California Trash Monitoring Playbook** and can be found in **Appendix B**.

Proposed Deliverables and Timeline

Table 4. Proposed tasks and deliverables and timeline for Trash in Estuaries and Wetlands.

Task/Deliverables	Estimated Completion Date
Field Deployment	Fall 2023
Data Submission	Spring 2024
Data Analysis	Summer 2024
Draft Report	Winter 2024
Final Report	Spring 2025

Epibenthic Marine Debris

Background and Objectives

Trash has become a focal point for many jurisdictions in southern California due to recent policies limiting the amounts of trash in the environment (i.e., CRWQCB 2007, CRWQCBLA 2015, SWRCB 2015). While most of these policies are specific to land-based trash, they ultimately have a direct impact on the amounts of trash that make it to the ocean and become marine debris. Most coastal studies quantifying marine debris have been localized, short-term surveys focused primarily on beach debris (Gabrielides et al., 1991; Moore et al., 2001; Ribic et al., 1992) and floating debris (Aliani et al., 2003; Barnes, 2002; Barnes and Milner, 2005; Day and Shaw, 1987). Few coastal studies have focused on epibenthic habitats of the continental shelf (Galgani et al., 1995, 1996; Keller et al., 2010; Moore and Allen, 2000; Stefatos et al., 1999; Watters et al., 2010), and only one, the Bight Regional Trash Survey, has been implemented on a regional and temporal scale. Regional and temporal assessments are necessary to assess the effectiveness of regulation, which requires information about the extent and magnitude of marine debris collected over sufficient time periods to determine trends.

Debris on the seafloor has been a part of the Bight survey since its inception in 1994. Trends over six Bight surveys (i.e., 1994, 1998, 2003, 2008, 2013, 2018) indicate that the amount (percent of area) of anthropogenic debris found on the continental shelf is not decreasing (**Figure 1**). In 2018, McLaughlin et al. (2022) estimated that anthropogenic debris was found in about 25% of the Bight with plastics being the largest component (about 21%). Continuing to monitor these trends is a crucial part of determining whether policies regarding trash are effective.

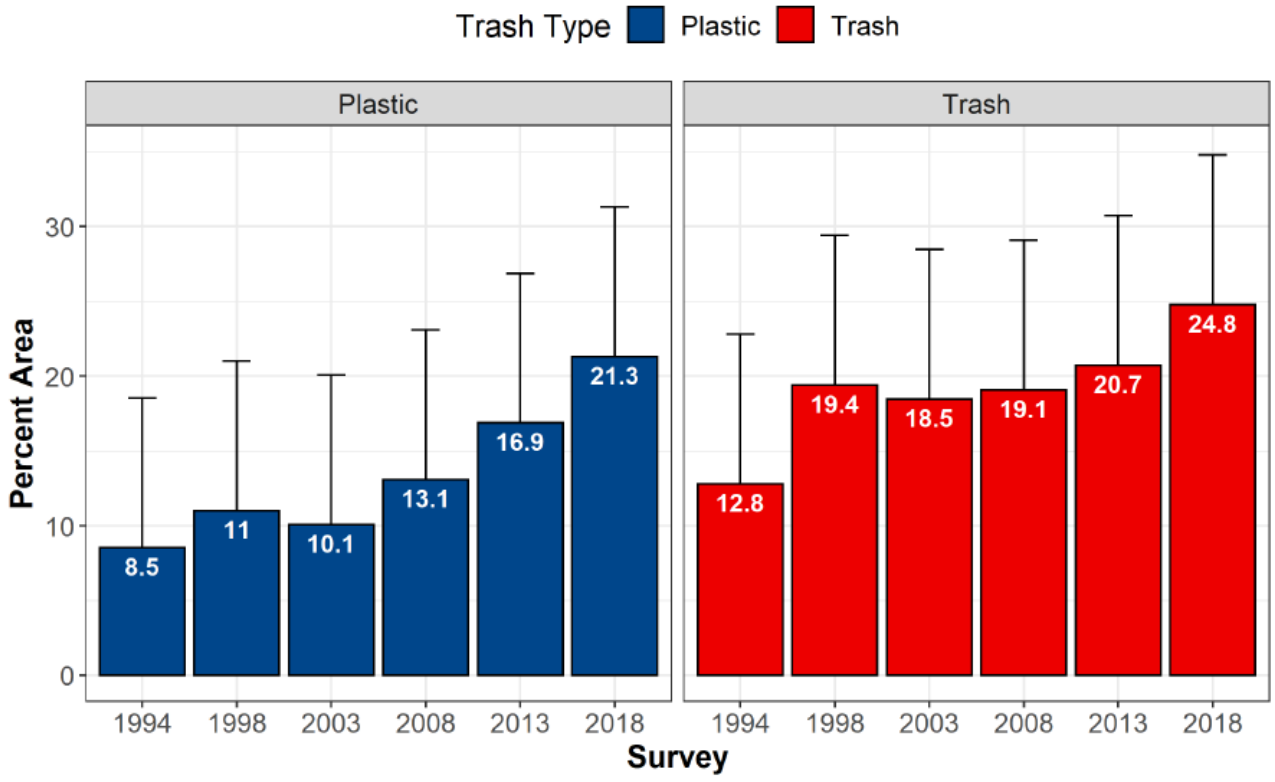


Figure 1. Percent area of the seafloor with plastic and trash for each Bight Regional Monitoring Survey.

The overall goals of the Epibenthic Debris Survey as part of the larger Bight Regional Survey are listed in **Table 5** and include looking at the extent and magnitude of debris as well as debris trends over all Bight surveys.

Table 5. Summary of management questions, study objectives, and intended data applications of the Bight '23 Regional Monitoring Epibenthic Debris Survey.

Management Question	Study Objective	Application
What is the extent and amount of trash on the seafloor?	Assess the spatial distribution and amount of trash on the seafloor	Determine the overall trash condition of the seafloor
What are the trends of trash types and amounts on the seafloor?	Assess the relative quantities of different trash types on the seafloor	Compare to previous Bight surveys to determine if conditions are improving or declining

Conceptual Approach

To collect epibenthic debris data the Trash and Microplastics Committee will collaborate and coordinate with the Sediment Quality Committee. Epibenthic debris data will be collected as a subcomponent of the trawl surveys conducted to obtain information on benthic fish and invertebrate communities. Field crews will process debris samples at the same time they process fish and invertebrates and will report the data to the Trash Committee. Results collected will be analyzed to answer the questions put forth by the Trash and Microplastics Committee (**Table 5**).

Standardized methods to enumerate epibenthic debris were developed during the first Bight Regional Survey in 1994 and have remained the same for every Bight Survey since. These methods are detailed below. Debris will be categorized, quantified, and recorded on standardized data forms (**Appendix C**).

Sampling and Analysis Methods

This study component is being leveraged over resources in place through the Sediment Quality Field Subcommittee, therefore the target population, sample frame, and site selection have been pre-determined by that workgroup. The target population for the 2023 Sediment Quality survey is all marine or marine-influenced, subtidal waters along the Southern California Bight to a depth of 500 m, excluding the Channel Islands. The sample frame for the epibenthic debris study will be the same as that for trawls and will include five strata used in previous Bight surveys. A target of 30 sites sampled has been set for each stratum. The trawl strata are as follows:

1. Bays
2. Inner Shelf
3. Middle Shelf
4. Outer Shelf
5. Upper Slope

Sites were selected randomly using a generalized random tessellation stratified (GRTS) procedure to ensure spatial balance among sampled sites, allow for inference into regional condition, avoid bias, and allow for extrapolation of the response to the entire stratum. Although sites were selected randomly, a systematic component was added to the selection process to minimize clustering of sample sites. The systematic element was accomplished by using an extension of the sampling design used in the Southern California Bight Pilot Program and in EPA's Environmental Monitoring and Assessment Program (EMAP) (Stevens 1997). A hexagonal grid is randomly placed over a map of the sampling area, a subsample of hexagons is chosen from this population, and samples are obtained at randomly selected sites within grid cells. The hexagonal grid structure ensures systematic separation of the sampling, while the random selection of sites within grid cells ensures an unbiased estimate of ecological condition.

Sample collection methods in the field will follow the Bight '23 Field Operations Manual during the summer of 2023 (July-September).

Trawls will be conducted using a semi-balloon otter trawl with a 7.6-m headrope (25 ft), 8.8-m footrope (29 ft), 3.8-cm (1.5 in) body mesh, and a 1.3-cm cod-end mesh (0.5 in). Trawls will be towed along isobaths at a speed-over-ground of approximately 0.8 to 1.0 m/second (or 1.5 to 2.0 kn) for 10 minutes. At the end of the prescribed trawl time, the net is retrieved and brought onboard the vessel. Any debris caught on the cable/doors/chain should be noted, but not included in the tally. The cod-end is opened, and the catch is deposited into a tub or holding tank. The criteria used to evaluate the success of any trawl includes making sure that proper depth, scope, speed, and distance (or duration) were maintained, whether the net was fouled (net tangled), and whether the catch shows evidence that it was on the bottom (e.g., rocks, epibenthic invertebrates, demersal fish).

The catch should initially be rough sorted into major categories (e.g., urchins, shrimp, other invertebrates, flatfishes, rockfishes, other fishes, debris). Trawl debris will be sorted for processing. Debris collected during any trawl will be quantified as well as qualified by recording the specific types of debris on the Bight '23 Trawl Debris Form. The larger categories on this form match those on the form used by the Stormwater Monitoring Coalition (SMC) for collecting debris information for land-based sources, in an effort to make comparisons of land-based trash versus ocean-based debris. For Bight '18, the form was modified to include Single Use Food Container and remove Pull Tab based on knowledge from the Debris Subcommittee participants with trawling programs. Similarly, the Bight '23 form was modified to include pandemic-related items such as masks and differentiates mylar and latex balloons. The major categories include Plastic, Glass, Metal, Miscellaneous Items, Marine Origin, and Terrestrial Origin. Items within these categories include those commonly found in previous surveys (**Appendix C**).

Types of items within each of these categories will be counted and recorded. If an item is not on the list, it will be placed in the appropriate "Other" category with a required comment made to describe the item. In the case of items that could fit into multiple categories, count the item in the category of its primary material, and document any of the other categories it would fit into in the comments field. Please note additional descriptive information regarding the debris such as brand names in the comments section for that item. For debris of marine or terrestrial origin, counts of each should be made; however, estimates are acceptable as well. For counts of ten or less, record the item count, for counts higher than ten record a qualifier in the estimate box based on the following categories: M for Moderate abundance (11-100 items); and H for High abundance (>100 items). In cases where counts were not easily made, include a comment explaining the reason(s) for the difficulty. No debris items will be weighed for Bight '23, but comments that better describe the debris such as estimated size (e.g., the size of a basketball), condition (e.g., decayed kelp frond in pieces), or type/species (e.g., *Macrocystis pyrifera*) are encouraged.

Proposed Deliverables and Timeline

Table 6. Proposed tasks and deliverables and timeline for Trash on the seafloor.

Task/Deliverables	Estimated Completion Date
Field Deployment	Summer 2023
Data Submission	Fall 2023
Data Analysis	Spring 2024
Draft Report	Summer 2024
Final Report	Spring 2025

Microplastics

Study Objective

The overall objective of the Bight '23 Microplastics Survey is to characterize the extent and magnitude of microplastics in the SCB coastal habitats. The primary question of interest is:

What is the extent and amount of microplastic contamination in the SCB?

This question seeks to quantify and characterize microplastic contamination in SCB sediments and shellfish as the first regional microplastic survey in coastal habitats of Southern California.

Background and Objectives

Microplastics, plastic particles <5 mm in size, are ubiquitous environmental contaminants, having been found in almost every type of habitat. In addition to their prevalence, microplastic exposure may cause negative health impacts in both aquatic organisms and humans. California's first piece of legislation that specifically applied to microplastics was passed in 2008, when strict regulations were placed on the manufacture, handling, and transport of pre-production plastic pellets. Then, in fall 2015, Assembly Bill 888 prohibited the sale of personal care products containing plastic microbeads – only a few months prior to enactment of a similar federal ban.

Most recently, in 2018, the California Legislature adopted a pair of bills that require the State to begin building microplastics management strategies for both drinking water and California's coastal ocean and estuaries. Senate Bill 1422 required the California State Water Resources Control Board to develop plans for measuring microplastic particles in drinking water. Senate Bill 1263 required the California Ocean Protection Council to adopt and implement a statewide strategy for lessening the ecological risks of microplastics to coastal marine ecosystems.

These bills have led to the standardization of analytical methods in drinking water (De Frond et al., 2022) as well as the anticipated standardization of methods to extract and measure microplastic particles in sediments, ambient water, and biological tissues (Thornton Hampton et al., 2023; Langknecht et al., 2023). In addition, a Statewide Microplastics Strategy was adopted by the Ocean Protection Council (OPC) in early 2022.

Microplastics have been excluded in previous Bight cycles due to the lack of standardized methods. However, now that method performance has been evaluated, this concern has been eliminated. Incorporating microplastics into the Bight '23 Regional Monitoring Program will allow for the regional assessment of microplastics in Southern California. This effort also directly aligns with the Statewide Microplastics Strategy, which emphasizes the need to leverage existing monitoring programs, such as Bight, to build a Statewide Monitoring Network for microplastics. The Bight '23 assessment of microplastics represents a foundational step to characterize the extent and magnitude of microplastic contamination in Southern California.

Conceptual Approach

The Bight '23 Microplastics Survey will assess microplastic contamination in sediment and bivalves by leveraging existing sampling efforts. To collect sediment samples, the Trash and Microplastics Planning Committee will coordinate with the Sediment Quality Committee and the Estuaries Committee. Field crews for the Sediment Quality Survey will collect sediment samples for microplastics analysis at up to 30 sites on the inner shelf, 10 sites within ports, 10 sites within embayments, and 10 sites within marinas between July and September 2023. The Estuaries Committee will collect sediment samples from up to 30 estuaries within the intertidal zone during estuarine assessments conducted in October 2023.

To collect shellfish, the Trash and Microplastics Planning Committee will coordinate with the Shellfish Committee. Mussels (*Mytilus* spp.) will be collected for microplastics analysis during the late summer (August-September 2024). At least five shellfish of each species present will be collected at each site to create a composite sample. At a pre-selected subset of sites, triplicate samples (i.e., three samples comprised of five pooled organisms each) will be collected to assess variation amongst individual samples.

Following collection, sediment and bivalve samples will be analyzed for microplastics using standardized methods evaluated during the SCCWRP Microplastics Interlaboratory Method Evaluation Study (Thornton Hampton et al., 2023; Langknecht et al., 2023). All suspected plastic particles ≥125 µm will be quantified and analyzed for size, morphology, and color. To confirm plastic particles and determine polymer type, up to 75 particles per sample will be randomly subsampled for spectroscopic analysis according to De Frond et al. (2023). Specifically, De Frond et al. suggest numbering all particles identified via microscopy and using a random number generator to determine which particles are selected for chemical identification. Results collected will be analyzed to answer the questions put forth by the Trash and Microplastics Committee (**Table 7**). For shellfish samples, 20-125 µm particles will be extracted and concentrated on filters for future potential analysis and described in **Appendix D**.

Table 7. Summary of management questions, study objectives, and intended data applications of the Bight '23 Regional Monitoring Trash survey in inland waterways.

Management Question	Study Objective	Application
What is the extent and amount of microplastic contamination?	Assess the amount and spatial distribution of microplastics	Establish baseline occurrence of microplastic contamination in the Southern California Bight
What are the types and relative amounts of microplastic contamination?	Assess the relative quantities of microplastic types (e.g., polymers, morphologies, sizes)	Identify most prevalent types of microplastics in the Southern California Bight

Sampling and Analysis Methods

Sediment Collection

Sediment collection for offshore sites (i.e., inner shelf, port, embayments, marinas) will occur as part of benthic chemistry sampling, with some modifications specific for microplastics. A 0.1 m^2 modified Van Veen grab will be used to collect sediment samples according to the Bight '23 Field Manual. Jars will be kilned at 500 °C for at least 1 hour to destroy all existing organic matter and rinsed with Microplastics Analysis Grade Water (i.e., MAG water, 1 µm filtered distilled water) prior to sample collection. The **top 5 cm** of the sediment will be collected using a stainless-steel scoop that has been pre-rinsed with MAG water immediately before collection. Sediment will be deposited into 16-ounce (~ 450 mL) pre-labeled glass mason jars. At half of the sites, a field blank will be collected using a 16-ounce pre-labeled glass mason jar pre-filled with 400 mL of MAG water. The jar will be placed as close as possible to the work area where sediment is being collected. The lid of the field blank jar will be removed when the top doors of Van Veen are opened, and immediately closed when the sediment sample has been successfully collected and the lid of the sample jar has been closed. A detailed standard operating procedure is provided in **Appendix D**.

Sediment collection for estuaries will occur as part of the Bight '23 estuary assessment. Sediment samples will be collected in the intertidal zone at low tide when the sediment is exposed. A 3" diameter aluminum pipe will be

used to collect the **top 5 cm** of sediment. Immediately following collection, the sediment will be deposited into a pre-labeled 16-ounce glass mason jar. All equipment and sample jars will be kilned and pre-rinsed with MAG water, as above, prior to sample collection. At half of the sites, a field blank will be collected in a similar manner as previously described for offshore sites. A detailed standard operating procedure is provided in **Appendix D**.

All sediment samples will be received by SCCWRP and redistributed to the appropriate laboratories for analysis. All sediment samples will be stored at 4 °C until analysis.

Field crews will be supplied with MAG water, sample jars, and field blanks. Field crews will be asked to take note of clothing worn while sampling as well as any other materials nearby (e.g., fraying ropes) that may possibly contribute to background contamination. This may be documented by taking a photo and sending it via email to leahth@sccwrp.org with the agency name and sampling day.

Sediment Analysis

Sediment samples will be analyzed according to protocols evaluated during the SCCWRP Interlaboratory Comparison Study (Langknecht et al., 2023; Cashman et al., 2022). Briefly, sediment samples will be homogenized at the receiving laboratory, and 1 g of sediment will be used for the determination of moisture content by weighing the material before and after complete drying. Microplastics will be extracted from 100 g of sediment (wet weight) via a series of density separations using sodium bromide (NaBr, ϱ=1.4 g/cm^3). Following extraction, particles >125 µm will be quantified and characterized for size (i.e., measured size of each particle), color, and morphology via visual (light) microscopy. Particles <125 µm will be discarded. A total of 75 particles will be randomly subsampled across size fractions >125 µm for spectroscopic analysis via Fourier Transform Infrared spectroscopy (FTIR) or Raman according to methods described in De Frond et al., (2023). Remaining sediment will be stored at 4°C for potential future analysis. A detailed standard operating procedure is provided in **Appendix D**.

Shellfish Collection

Shellfish samples will be collected at low tide when their shells are closed. Each field crew will be required to collect one field blank at one randomly selected site as a precaution in case sample contamination in the field is suspected. Five shellfish of each species will be collected from each site, depending on the season (see Conceptual Approach section for details regarding seasonal component of shellfish collection). Each individual shellfish will be wrapped in pre-kilned heavy-duty foil and stored in a cooler on ice or at 4 °C until samples are received by SCCWRP. Upon receipt, shellfish will be shucked in the SCCWRP Chemistry Laboratory under clean conditions (e.g., HEPA filtration, staff wearing cotton lab coats, under hood, etc.). Tissues will be divided amongst 3 polypropylene jars (5 tissues/jar). Samples will be stored at –20 °C and redistributed to the appropriate laboratories for analysis.

Shellfish Analysis

Shellfish samples will be analyzed according to protocols evaluated during the SCCWRP Interlaboratory Comparison Study (Thornton Hampton et al., 2023). Briefly, microplastics will be extracted by digesting composite tissues in 20% potassium hydroxide (KOH) at 45 °C. Following tissue digestion, the contents of the sample jars from each site will be combined, size fractioned, and vacuum filtered. Particles >125 µm will be quantified and characterized for size (i.e., measured size of each particle), color, and morphology via visual microscopy. Particles <125 µm will be reserved for potential future analysis. A total of 75 particles will be randomly subsampled across size fractions >125 µm for spectroscopic analysis via Fourier Transform Infrared spectroscopy (FTIR) or Raman according to methods described in De Frond et al. (2023). A detailed standard operating procedure is provided in **Appendix D**.

Calculating Detection Limits for Microplastic Samples

The minimum detectable amounts (MDA) will be calculated for each laboratory for both sediment samples and shellfish according to Lao & Wong (2023). The MDA is defined as, "…the minimum number of microplastic particles that must be present in a sample to give a specified power, (1-β), and estimates the critical value (Lc): the minimum significant value to discriminate net count (Ns) against background count (Nb), and ultimately decide if the analyte is detected or not detected" (Lao & Wong 2023). MDAs will be calculated for total particle count, each size fraction, and for fibers vs. non-fibers for each matrix.

Proposed Deliverables and Timeline

Table 8. Proposed tasks and deliverables and timeline for microplastics in sediment and shellfish.

Task/Deliverables	Estimated Completion Date
Field Deployment	Summer 2023 (sediment)/Fall 2024 (shellfish)
Data Submission	Fall 2025 (sediment)/Spring 2026 (shellfish)
Data Analysis	Winter 2025 (sediment)/Summer 2026 (shellfish)
Draft Report	Summer 2026
Final Report	Winter 2026

Quality Assurance and Quality Control

Overview

The primary goal of the QA Plan and related quality control activities (collectively QA/QC) is to ensure that the data generated in the Bight '23 program are comparable among participants. Many different organizations will be participating in the collection and analysis of samples in Bight '23; encouraging and maintaining consistency in field and laboratory operations and ensuring data comparability will be critical to success of the survey.

General Approach to Quality Assurance

The QA program for Bight '23 consists of two distinct but related activities: quality assurance and quality control. Quality assurance (QA) includes design, planning, and management activities conducted prior to implementation of the survey to ensure that the appropriate kinds and quantities of data will be collected. The goals of quality assurance are to ensure: 1) field collection, processing, and laboratory analytical techniques will be applied consistently and correctly; 2) the number of lost, damaged, and uncollected samples will be minimized; 3) the integrity of the data will be maintained and documented from sample collection to entry into the data record; 4) all data will be comparable; and 5) results can be reproduced.

Quality control (QC) activities are implemented during the data collection phase of the survey to evaluate the effectiveness of the QA activities. QC activities ensure that measurement error and bias are identified, quantified, and accounted, or eliminated, if practical.

Measurement Quality Objectives

The effectiveness of QA/QC activities are determined via quantitative measures known as Measurement Quality Objectives (MQOs). An MQO for accuracy is not applicable as internal standards are not available for trash or microplastics. However, MQOs for precision have been determined to ensure comparability amongst analytical laboratories (**Table 9**). The MQO for completeness is 90% for each measurement process. The sampling design for the survey is sufficiently redundant to absorb the loss of up to 10% of the samples without compromising the goals of the program, provided that the lost samples are not concentrated in a single subpopulation of interest. Redundancy was incorporated at this level because monitoring programs of this size typically lose as many as 10% of samples as a result of logistical difficulties or failure to achieve quality control criteria.

Table 9. Measurement quality objectives for Bight '23 Trash and Microplastics element.

Indicators	Precision	Completeness
Trash		
Inland Streams	-	90%
Estuaries	-	90%
Epibethic	-	90%
Microplastics		
Sediment	Within 20% of Grand Mean Total Particle Recovery	90%
Shellfish	Within 20% of Grand Mean Total Particle Recovery	90%

Trash

Quality assurance activities for trash assessments are described in the Bight ’23 Quality Assurance Manual. Relevant activities include the documentation of all protocols and field data sheets in the Bight ’23 Field Operations Manual in addition to a series of meetings with field crews to ensure that participants are familiar with all procedures to achieve MQOs.

For epibenthic marine debris, field audits will be conducted for each vessel to ensure protocols are properly followed as a quality control measure. Trawling must also adhere to the criteria outlined in the Bight ’23 Field Operations Manual and the Bight ’23 Quality Assurance Manual.

Microplastics

Sediment and shellfish will be collected as part of the Bight ’23 Survey for microplastics analysis. In addition to the quality assurance and control procedures outlined in the Bight ’23 Quality Assurance Manual, an Interlaboratory Comparison Study will be conducted prior to sample analysis and background contamination will be extensively characterized during sample collection and processing.

Interlaboratory Comparability

Prior to analyzing sediment or shellfish samples for microplastics collected in the Bight ’23 Survey, all participating laboratories must participate in the interlaboratory comparison exercise to ensure comparability of results. Each participating laboratory will receive one microplastic-spiked sample and one blank sample for each matrix (i.e., sediment and/or shellfish) to be processed analyzed prior to samples collected during the Bight ’23 Survey. Spiked samples will consist of either sediment or tissue spiked with a known quantity and composition of microplastic particles. Details regarding the quantity and composition of the microplastic spike will be withheld from participating laboratories to eliminate bias. Laboratories will be required to use the previously selected methods for each matrix (see Appendix D) and submit their results to the Bight ’23 Data Portal (see Appendix E). Successful completion of the interlaboratory comparison exercise will be evaluated based on three criteria: 1) recovery of microplastic particles, 2) method blank contamination, and 3) comparability among laboratories:

- Total particle recovery for spiked microplastic samples must exceed the lower quartile for total particle recovery > 20 µm reported during the Microplastics Interlaboratory Method Comparison Study (Thornton Hampton et al., 2023). Specifically, total particle recovery must be greater than 31% for sediment and 43% for shellfish samples.

- Method blank samples will include the matrix of interest (i.e., sediment or tissue) without any spiked microplastic particles. Method blank particle counts must not exceed the upper quartile detected in blank samples during the Microplastics Interlaboratory Method Comparison Study (Thornton Hampton et al., 2023). Specifically, total particle counts must be less than 23 particles for sediment and 110 particles for tissue.

- Comparability of the labs will be based on total particle recovery of the spiked samples. Participating laboratories must demonstrate a total particle recovery of the spiked microplastics within 20% relative difference of the grand mean (i.e., mean total particle recovery across all participating laboratories). This value (i.e., 20% relative difference) was derived from the Microplastics Interlaboratory Method Comparison Study where the mean relative percent difference amongst labs relative to the grand mean was 15 and 18% for sediment and tissue, respectively (Thornton Hampton et al., 2023).

- Laboratories that fall below minimum particle recovery limits, exceed maximum particle counts in blank samples, or otherwise do not meet comparability criteria will be subject to a review of test procedures and possible re-analysis of new method blank and/or spiked samples.

- All participating laboratories will be required to submit Interlaboratory Comparability results data to the Bight '23 Data Portal (https://bight.sccwrp.org) by Friday, January 19, 2024.

Surrogate Recovery

Particle recovery will be assessed in field collected samples by spiking all samples with known quantities and types of microplastics (Table 10). The smallest and largest size fractions will be spiked with microspheres and fibers, varying in color by size and morphology. Recovery of these particles in spiked samples will be reported upon data submission.

Table 10. Particles to be used for surrogate recovery estimates.

Particle	Morphology	Size (µm)	Polymer	Density (g/cm^3)	Color	Manufacturer and Item Number	Number of Spiked Particles
1	Sphere	600-700	PE	1.00	Blue	Cospheric; BLPMS-1.00 600-700µm	10
2	Sphere	300-355	PE	0.98	Green	Cospheric; GPMS-0.98 300-355 µm	10
3	Fiber	>500	PET	~1.38	Orange	-	10

Abbreviations: Polyethylene (PE); Polyethylene terephthalate (PET).

Documentation of Background Contamination

Background contamination is a notable concern when collecting and analyzing microplastic samples. Eliminating background contamination is impossible, but specific QA/QC procedures will be implemented to minimize and characterize any contamination that may occur:

- Contamination during sediment sample collection will be assessed using field blanks. Field blanks will consist of sampling jars filled with 250 mL of MAG water. Field blank jars will be opened during sample collection and placed near the working area to capture particles that may be deposited when sediment is being sampled. Field blanks are assigned to 50% of the sites and will be processed and analyzed according to the sample protocols as for true samples (Appendix D).

- In addition to field blanks, field crews will take a group photo on each sampling excursion to document clothing (a potential source of contamination due to the shedding of fibers). In addition, field crews have been instructed to be cautious of materials on vessels that may contaminate samples and to remove them from the sampling area if possible (e.g., fibrous floor mats, fraying ropes, etc.).

- Contamination during sample processing and analysis will be assessed using method blanks. Method blanks will be processed and analyzed with every batch of true samples using previously selected methods for each matrix (see Appendix D). Method blanks will consist of 250 mL MAG water in containers identical to true samples.

- Participating laboratories will be asked to document their background mitigation procedures during data submission (Appendix E).

Appendix A: Data Sheets for Trash in Inland Waterways

Methods for Assessing Trash in Inland Streams

1. Event Preparation. Field teams are recommended to perform event preparation in the office before beginning field activities. The recommended pre-monitoring information should be reviewed:

1. Time restrictions

2. Driving directions to site

3. Verification of the access route to site, including which gates to enter, access ramps into engineered channels, and the walkways or rails

4. Parking locations and whether the site has time restrictions for parking

5. Familiarization with the site map

6. An inventory of the field items needed and whether additional supplies need to be procured

2. Gather Standard Equipment. Suggested equipment for field teams includes the following:

- Clipboard
- Maps
- Pens/pencils
- GPS
- Camera
- Measuring Rangefinder or transect tape
- Survey flags
- Waders/Rain boots
- Gloves
- Grabber tools
- Sunblock
- Hand sanitizer
- Insect repellant
- Drinking water
- Field forms (see **Appendix A**)

3. Set Up the Assessment Area. Locate the target location (latitude and longitude) if one is given and use a survey flag mark it as the starting point (most downstream point (A); **Figure 1A**). If this is a targeted site, choose a starting point. Then measure 30 m from the starting point upstream and mark the upstream point (C) with a flag. The midpoint (B) is halfway between the upstream (C) and downstream (A) points. Ropes, cones, or measuring tapes can be used to delineate the assessment area.

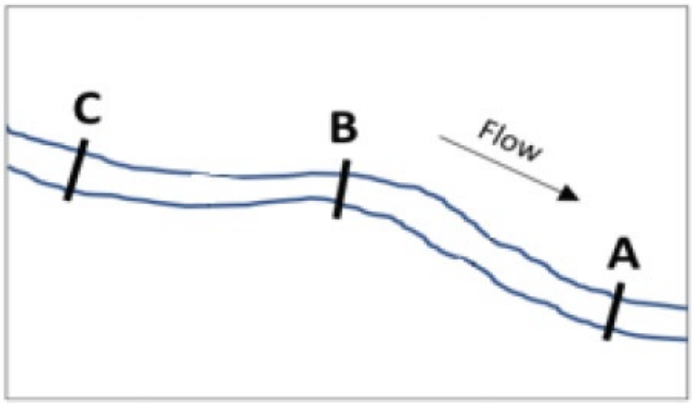


Figure 1A. Representative diagram of the assessment area (California Trash Monitoring Playbook).

Next, the measurements for bankfull and wetted width must be determined. The teams measure the wetted and bankfull widths of the stream shown in **Figure 2A** using a measuring tape (a rangefinder may be used for larger streams) and record the measurement on the **General Site Information** section on the **Riverine Site Information Data Sheet** (**Appendix A**). The assessment area width extends to the bankfull width of the stream. Evidence for bankfull locations includes topography, vegetation, sediment type, changes in bank slope, and location of water stains on concrete or bedrock. Bankfull width is determined by estimating the maximum water inundation in a one- to two-year flood event (Ode et al., 2016). Walk beyond the wetted width of the stream to look for evidence of one- to two-year flood events and that is the bankfull width. To measure this distance lay the measuring tape along the contour of the river to determine the total length (this differs from other methods that measure bankfull width as the taught distance of the measuring tape from one bank to the other). Measuring the bankfull width in this way will give the most accurate area estimate. The assessment area extends from the thalweg (line of lowest elevation within a water course) to the bankfull width on the left and right bank (face downstream to determine left or right bank). An example of the wetted and bankfull width measurements in the Los Angeles River are provided in **Figure 3**.

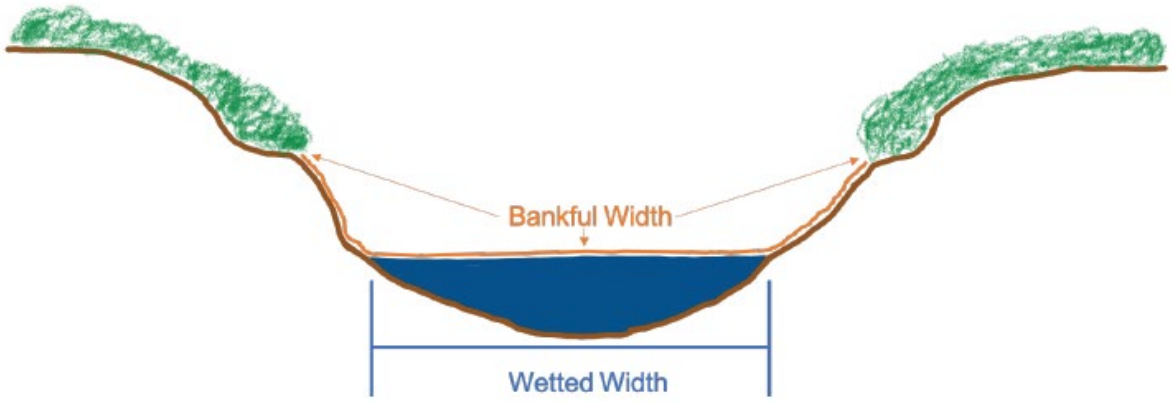


Figure 2A. Example stream cross-section wetted width and bankfull width (California Trash Monitoring Playbook).

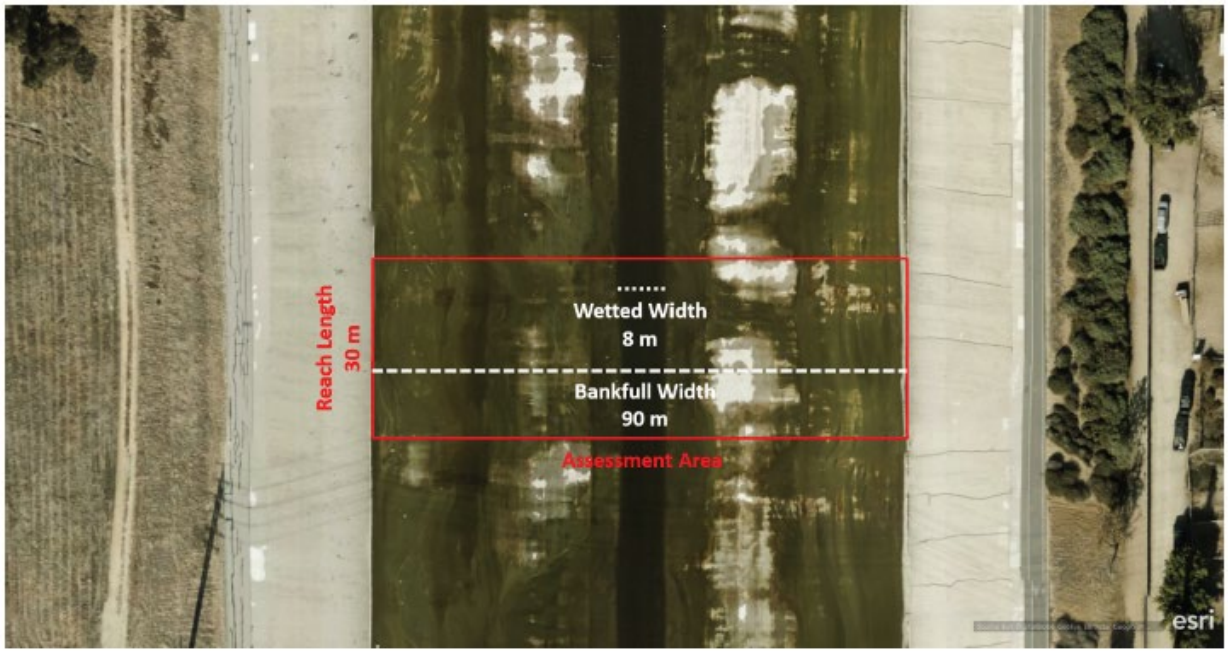


Figure 3A. An example of measuring bankfull width and wetted width in the Los Angeles River.

4. Record the Site Information and Assessment Area Dimensions

Fill out the **General Site Information** section on the **Riverine Site Information Data Sheet** (**Appendix A**). Record the station Identification (ID), start and stop time, latitude and longitude (in decimal degrees to at least 5 places) using a geospatial mobile app, and the datum (geographic information system [GIS] projection used as a point of reference for the site locations). Document the members of the field crew conducting the survey as well as a brief River/Site Description and the location of the Watershed site. Describe access to the site based on ease of access from both the right and left banks. Channel Type is based on the channel substrate and consists of natural (no apparent modifications made to the stream bed), earthen (natural stream bed that has been modified), and concrete. Finally, record the type of site being surveyed (probabilistic versus targeted) and whether the stream is flowing.

Site measurements should also be completed at this time. All measurements should be taken in meters and recorded on the **Assessment Area** section on the **Riverine Site Information Data Sheet** (**Appendix A**). These measurements are important to determining the area of the assessment site to allow for determination and comparison of trash densities both spatially and temporally. Transect A is the downstream point of the assessment site, Transect B is the midpoint, and Transect C is the upstream point. (If prevented from measuring the area at its midpoint due to safety or access constraints, select a nearby transect or omit the midpoint measurement.) It is also important to note whether the trash is being picked up during the assessment, so if a site is visited again an estimate of accumulation rates can be made.

5. Record Assessment Area Photographs

Photograph trash conditions during each assessment. Take a minimum of four photographs from the thalweg location at each site beginning at the upstream boundary (looking downstream), at the center area looking upstream and downstream, and at the downstream boundary (looking upstream) of the assessment area. Additional photographs may be taken to document site conditions or selected trash items. Each Photograph ID

should use the following naming convention: Unique Site ID-Photo Location-Survey Date (month.day.year), e.g., SMC00000B-Down-06.12.2018. Complete the field form in **Table 1A** to record the metadata on the images taken.

Table 1A. Photograph documentation form for the visual observation method.

Segment Photograph	View Direction	Photograph ID
Bottom (A)	Upstream	
Middle (B)	Upstream	
	Downstream	
Top (C)	Downstream	
Other Photos	Misc. 1	
	Misc. 2	

6. Determine the Locations of Storm Drain Outfalls and Homeless Encampments within the Assessment Area

Record the number and size of stormwater outfalls (>18 inches) in the assessment area and record this information in the **Stormwater Outfalls/Encampments** section on the **Riverine Site Information Data Sheet** (**Appendix A**) for each outfall present in the assessment area. Outfalls include any pipes or discharge areas from outside of the river/stream. Outfall categories are as follows: 18–24 inches, 25–36 inches, 37–48 inches, and >48 inches. Record if there is trash at the outfalls and the amount of trash present. Trash at the outfalls is the trash that is in the immediate vicinity of, and appears to have come out of, the outfall. Trash count (number of items) categories are as follows: <10, <50, <100, and ≥100.

Determine whether there is a homeless encampment in the assessment area or within 200 meters of the assessment area, either upstream or downstream. Record this information in the **Stormwater Outfalls/Encampments** section on the **Riverine Site Information Data Sheet** (**Appendix A**).

7. Record Visual Observations (Tier 1, Riverine Visual Observation Method)

This method must be performed by at least two crew members (one being the Field Crew Supervisor) to minimize potential subjectivity in the final score for the assessment area. The Field Crew Supervisor first walks the entire assessment area and scores the site based on a "first impression" of the amount of trash observed.

Walk on both banks and within or near the site (where feasible) to observe trash throughout the assessment area. Feasible conditions refer to flow conditions that allow the stream to be wadable, in addition to conditions that would avoid impacts on migratory nesting birds and spawning fish. Trash that is visible outside of the assessment area will be included in the trash condition score but will be noted on the field form.

Trash levels are scored based on the following scale:

- Low (1–3)
- Moderate (4–6)
- High (7–9)
- Very High (10–12)

Descriptions of the categories may be found on the **Riverine Trash Condition Categories and Scoring System Form** (**Appendix A**). **Figures 4A** through **7A** provide photographs representing the amounts of trash found in each Trash Condition Category. Photographs were obtained from the Bay Area Stormwater Management Agencies Association Receiving Water Trash Monitoring Program Plan for the San Francisco Bay Region (BASMAA 2018).

Selection of the number within the Trash Condition Category should be based on the description provided on the **Riverine Trash Condition Categories and Scoring System Form** (**Appendix A**). The uppermost description within the Trash Condition Category corresponds to the smallest number within the Site Score (e.g., a value equal to 1 represents the description "Effectively No or Very Little Trash"). Similarly, the lowermost description within the Trash Condition Category corresponds to the largest number within the Site Score. If trash occurs in piles in the assessment area, imagine the trash spread out through the entire area for assigning a score.

Figure 4A. Example of "Low" Trash Condition Category.

Figure 5A. Example of “Moderate” Trash Condition Category.

Figure 6A. Example of "High" Trash Condition Category.

Figure 7A. Example of "Very High" Trash Condition Category.

8. Collect Trash Items and Record the Number of Each Item Found (Tier 4, Riverine Quantitative Tally Method)

Begin on either the right or left bank, walking slowly while visually scanning for trash. Scan an area within a shoulder-width zone to avoid missing small or partially covered items and continue the process until the entire assessment area has been walked. A larger scan width may be used at sites with little or no vegetation. The recommended approach for field teams is to adopt a systematic scan approach while walking in the assessment area. An approach that focuses on walking a continuous S-shaped path over the assessment area, as shown by the example of the lawnmower pattern in **Figure 8A**, provides a systematic approach for observing trash and minimizing opportunities for missing small or difficult-to-observe trash pieces.

Trash is divided into nine major categories, including plastic, miscellaneous, fabric and cloth, biodegradable, biohazard, construction, glass, large, and metal. From there each category is broken into different items. For example, the plastic category is broken down into Bag - reusable, Bag - recyclable, Bag pieces, Balloons, etc. Counts of each item within a category should be measured by tallying them on the **Riverine Quantitative Tally Method Datasheet** (**Appendix A**). If multiple pieces of trash in the same approximate area appear to come from the same item (i.e., as broken-off fragments), count the pieces as one item; however, if the pieces appear to come from different items, count them separately. For example, if multiple pieces of plastic bags are present and they all are the same color and thickness, and all appear to be weathered similarly, count the item as one. Otherwise, the pieces should be counted separately.

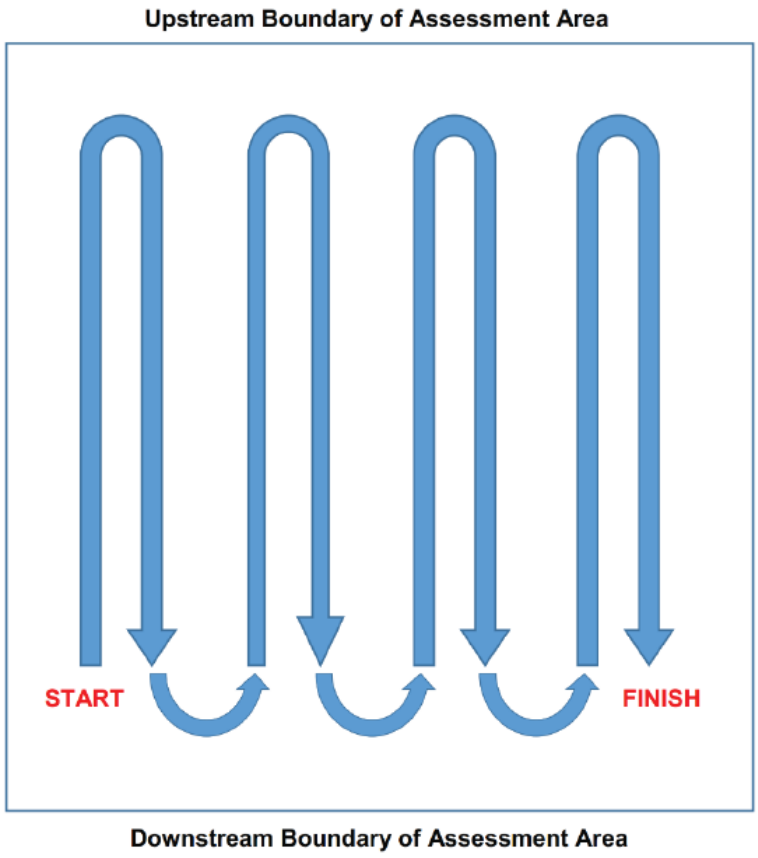


Figure 8A. Example "Lawnmower Pattern" for systematically walking assessment area for riverine surveys.

The count associated with a subset of items (list below) may be estimated if there are more than 11 pieces at a site using the following categories: Medium (M) = 11-100 pieces and High (H) ≥ 101 pieces.

Items That May Be Estimated on the **Quantitative Tally Method Datasheet** (**Appendix A**):

- Bag pieces
- Foam pieces
- Soft plastic pieces
- Wrapper/wrapper pieces

- Yard waste/leaf piles
- Glass pieces
- Aluminum foil pieces

Trash counts are recorded on the field form based on the specific type of material and amount present, either by estimating according to the listed items above or by counting the individual items. If estimates are used, then it is recommended to use the number marking the lowest range of the estimate for the overall count.

All biohazards and hazard waste need to be separated and disposed of according to conventional practices (e.g., syringes with needles need to be disposed into Sharps containers). Garbage bags should not be filled with more than 40 to 50 pounds of material. If material contains sharp or large objects, the material should be "double bagged," as necessary. Multiple garbage bags may be needed at each assessment site. Large items that cannot be removed by the field team should be reported to the landowner (e.g., local City government officials) for removal by City staff or their contractor.

Riverine Site Information Data Sheet

General Site Information

Station ID: _____ Date: _____

Start Time: _____ End Time: _____

Start Latitude: _____ Start Longitude: _____ Datum: ☐ NAD 83 ☐ WGS 84

End Latitude: _____ End Longitude: _____ ☐ Other

Field Crew: _____

_____

River/Site Description: _____ Watershed: _____

Access:

Left Bank (circle one): Easy Moderate Hard Right Bank (circle one): Easy Moderate Hard

Channel Type (check all that apply):

☐ Natural ☐ Earthen ☐ Concrete ☐ Rip Rap ☐ Other

Type of Site: ☐ Probabilistic ☐ Targeted Is stream flowing? Yes / No

Assessment Area

Reach Length: _____ ft / m (circle one) Circle One:

Wetted Width Transect A _____ Transect B _____ Transect C _____ ft / m

Bankfull Width Transect A _____ Transect B _____ Transect C _____ ft / m

Assessment Width Transect A _____ Transect B _____ Transect C _____ ft / m

Trash picked up during assessment? Yes / No

Stormwater Outfalls/Encampments

Number of stormwater outfalls in the assessment area > 18" in diameter:

18-24" _____ 25-36" _____ 37-48" _____ >48" _____

Trash at outfalls? Yes / No

Amount of trash present (number of pieces): <10 <50 <100 >100 (circle one)

Homeless encampment within 200 meters of assessment area? Yes / No (circle one)

Comments/Notes

Riverine Trash Condition Categories and Scoring Form

	Trash Condition Category											
	Low			**Moderate**			**High**			**Very High**		
Description	•Effectively no or very little trash •On first glance, little or no trash is visible •Little or no trash is evident when streambed and stream banks are closely examined for litter and debris •One individual could easily remove all trash observed within 10 minutes (100 ft AA*) or 30 minutes (300 ft AA)			•Predominately free of trash except for a few littered areas •On first glance, trash is evident in low levels •After close inspection, small levels of trash are evident in stream bank and/or streambed •On average, all trash could be removed by two individuals within 10 to 20 minutes (100 ft AA) or 30 minutes (300 ft AA) •Approximately 2-3 times more trash than the low condition category			•Predominately littered except for a few clean areas •Trash is evident upon first glance in moderate levels along streambed and banks •Evidence of site being used by people: scattered cans, bottles, food wrappers, plastic bags, etc. •On average, would take a more organized effort (more than 2 people, but less than 5) to remove all trash from the area. Removal of trash would take 10 to 30 minutes (100 ft AA) or 30 mins to 2 hours (300 ft AA) •Approximately 2-6 times more trash than the moderate condition category			•Trash is continuously seen throughout the assessment area •Trash distracts the eye on first glance •Substantial levels of litter and debris in streambed and banks •Evidence of site being used frequently by people (e.g., many cans, bottles, food wrappers, plastic bags, clothing, piles of garbage and debris) •On average, would take a large number of people (more than 5) during an organized effort to remove all trash from the area. Removal of all trash would take >40 minutes (100 ft AA) or >2 hours (300 ft AA) •Approximately >2 times more trash than the high condition category		
Site Score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

Photo Documentation

Segment	Location	Photograph ID
Bottom (A)	Upstream	
Middle (B)	Upstream	
	Downstream	
Top (C)	Downstream	
Other Photos	Misc 1	
	Misc 2	
	Misc 3	

*AA = Assessment Area

Riverine Quantitative Tally Method Datasheet

Station ID: _____ Date: _____ Initials: _____

Plastic	Tally Marks	Total
Bag - reusable		
Bag - single use		
Bag Pieces*		
Balloons – Latex		
Balloons - Mylar		
Beverage Bottles		
Bottles		
Chip Bags		
Cigar Tips		
Cigarette Butts		
Cigarette - Electronic		
Container Cap/Pieces		
Cups		
Foam Cups		
Foam Food Containers		
Foam Other Containers		
Foam Pieces/Balls/Pellets/Peanuts*		
Foam Plate		
Hard Plastic Container		
Hard Plastic Pieces		
Lid		
Lighters		
Pens/Markers		
Pipe		
Plates		
Straw Wrapper		
Single Use Container		
Soft Plastic Pieces*		
Straw/Stirrer		
Tarp		
Tobacco Wrapper/Pieces		
Trash Bag		
Wrapper/Wrapper Pieces*		
6-Pack Holder		
Plastic Other		

Fabric and Cloth	Tally Marks	Total
Natural (Cotton, Wool)		
Shoes		
Synthetic Fabric		
Tent/Sleeping Bag		
Fabric Other		

Large	Tally Marks	Total
Furniture/Appliances		
Garbage Bags of Trash		
Shopping Carts		
Tires		
Large Other		

Biodegradable	Tally Marks	Total
Food Waste		
Paper/ cardboard		
Yard Waste/Leaf piles*		
Biodegradable Other		

Biohazard	Tally Marks	Total
Condoms		
Dead Animals		
Human Waste/Diapers/TP		
Latex Gloves		
Mask – Single Use		
Mask – Cloth		
Medical waste		
Pet Waste		
Biohazard Other		

Construction	Tally Marks	Total
Bricks		
Concrete/Asphalt		
Fabricated Wood		
Rebar		
Construction Other		

Glass	Tally Marks	Total
Glass Bottles		
Glass Pieces*		
Glass Other		

Metal	Tally Marks	Total
Aluminum Foil pieces*		
Aluminum or Steel Cans		
Auto Parts		
Batteries - Alkaline		
Batteries - Lithium		
Metal Bottle Caps		
Metal Pipe/Bar Segments		
Nails, Screws, Bolts, etc.		
Spray Paint Cans		
Wire (barb, chicken, etc.)		
Metal Other		

Miscellaneous	Tally Marks	Total
Ceramic Pots/Shards		
E-waste		
Foam rubber		
Hose/Hose pieces		
Rubber/Rubber pieces		
Sports Balls		
Waxed Paper Cups/Plates		
Misc. Other		

GRAND TOTAL:

***These items may be binned if abundance is greater than 10 pieces as follows:**

M = 11-100 pieces

H ≥ 101 pieces

Appendix B: Data Sheets for Trash in Estuaries and Wetlands

Methods for Assessing Trash in Estuaries and Wetlands

Materials

1. Clipboard
2. Station polygon maps
3. Pens/pencils
4. GPS
5. Camera
6. 100-m transect tape
7. Survey flags
8. Gloves
9. $1m^2$ quadrat
10. Field forms

Field Methods – Within vegetation transects

Set Up the Transects. Trash will be assessed within quadrats along the vegetation transects, as delineated in SOP 11, Figure 1.

a. At a minimum, trash should be tallied within quadrats at station #2. If time permits, trash should be tallied across all quadrats and stations.

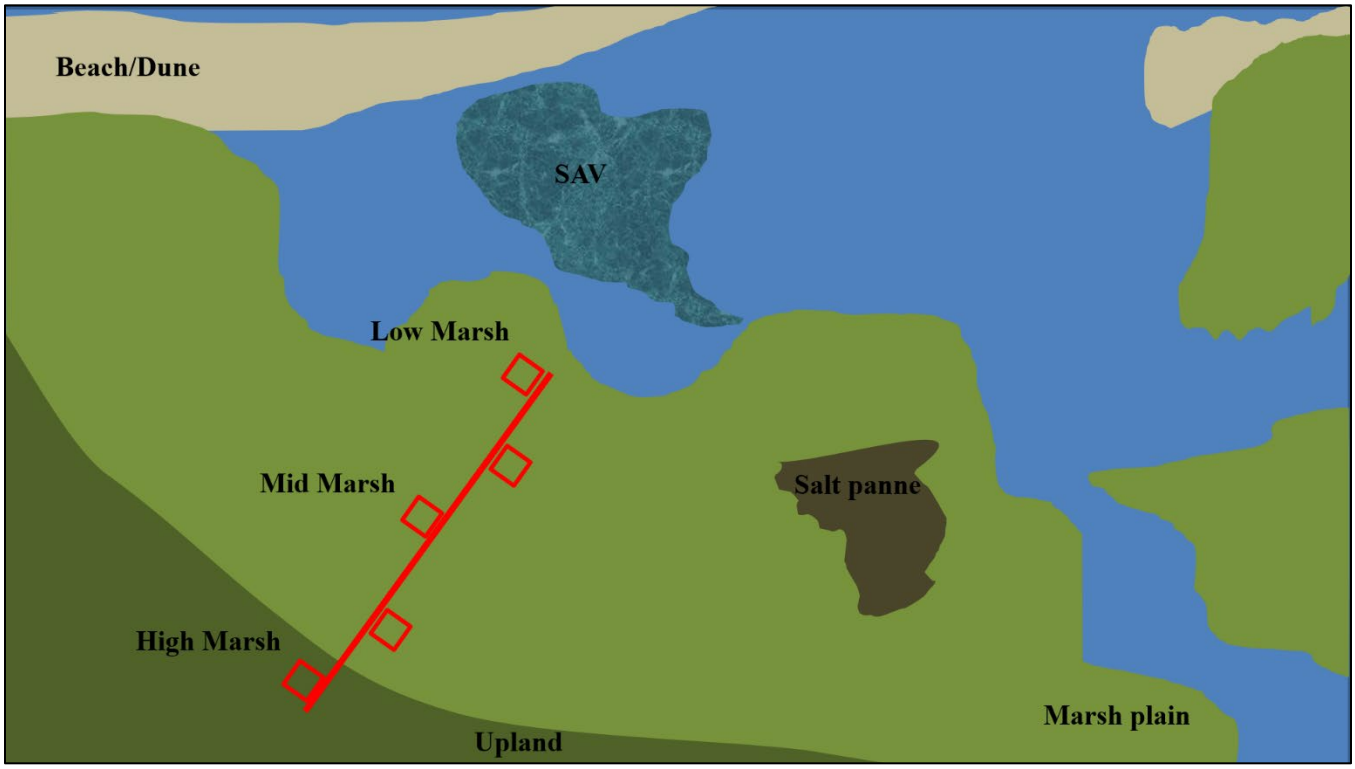


Figure 1. Representative diagram of the transect sampling area (adapted from the California Estuary Marine Protected Area Monitoring Program Protocol for March plain vegetation epifauna surveys).

1. **Record the Station and Sampling Area Information for Transects**

Fill out the **General Site/Station Information** section on the **Estuary Site Information Transects Data Sheet.** Record the station Identification (ID), start and stop time. Document the members of the field crew conducting the survey. Record the transect number, transect length, and total number of quadrats across the transect.

For each quadrat, record the latitude and longitude (in decimal degrees to at least 5 places) at the corner of the quadrat. Indicate the habitat type the quadrat represents (low marsh, mid marsh, high marsh, wrack line, etc.), whether the quadrat crosses the wrack line, and whether or not trash is found.

This method should be completed by two people – one person calling out trash and one person recording it on the tally datasheet. The recorder should familiarize themselves with the datasheet prior to surveying.

2. **Tally Trash Items for Transects**

Within each vegetation quadrat along the transect, tally the number and type of trash items found in each quadrat using the **Estuary Quantitative Tally Method Transects Datasheet.** If no trash items are found, this is indicated on **Estuary Site Information Transects Data Sheet** and the **Estuary Quantitative Tally Method Transects Datasheet** does not need to be completed.

Trash is divided into nine major categories, including plastic, miscellaneous, fabric and cloth, biodegradable, biohazard, construction, glass, large, and metal. From there each category is broken into different items. For example, the plastic category is broken down into Bag - reusable, Bag - recyclable, Bag pieces, Balloons, etc. Counts of each item within a category should be measured by tallying them on the **Estuary Station Quantitative Tally Method Datasheet**. If multiple pieces of trash in the same approximate area appear to come from the same item (i.e., as broken-off fragments), count the pieces as one item; however, if the pieces appear to come from different items, count them separately. For example, if multiple pieces of plastic bags are present and they all are the same color and thickness, and all appear to be weathered similarly, count the item as one. Otherwise, the pieces should be counted separately.

The count associated with a subset of items (list below) may be estimated if there are more than 11 pieces at a site using the following categories: Medium (M) = 11-100 pieces and High (H) ≥ 101 pieces.

Items That May Be Estimated on the **Estuary Station Quantitative Tally Method Datasheet:**

- Bag pieces
- Foam pieces
- Soft plastic pieces
- Wrapper/wrapper pieces
- Yard waste/leaf piles
- Glass pieces
- Aluminum foil pieces

Trash counts are recorded on the field form based on the specific type of material and amount present, either by estimating according to the listed items above or by counting the individual items. If estimates are used, then it is recommended to use the number marking the lowest range of the estimate for the overall count.

Field Methods – Station-wide trash

1. **Record Visual Observations within Station (Tier 1, Adapted Riverine Visual Observation Method)**

This method must be performed by at least two crew members (one being the Field Crew Supervisor) to minimize potential subjectivity in the final score for the station area. The Field Crew Supervisor first walks the entire sampling area (the entire station polygon) and scores the site based on a “first impression” of the amount of trash observed.

Trash levels are scored based on the following scale:

- Low (1–3)
- Moderate (4–6)

- High (7–9)
- Very High (10–12)

Descriptions of the categories may be found on the **Estuary Station Trash Condition Categories and Scoring System Form** (**Figure 2**). **Figures 3-5** provide photographs representing the amounts of trash found in each Trash Condition Category. Selection of the number within the Trash Condition Category should be based on the description provided on the **Estuary Station Trash Condition Categories and Scoring System Form**. If trash occurs in piles in the assessment area, imagine the trash spread out through the entire area for assigning a score.

	Trash Condition Category											
	Low			**Moderate**			**High**			**Very High**		
Description	•Effectively no or very little trash •On first glance, little or no trash is visible •Little or no trash is evident when site is closely examined for litter and debris •One individual could easily remove all trash observed within 10 minutes			•Predominately free of trash except for a few littered areas •On first glance, trash is evident in low levels •After close inspection, small levels of trash are evident in site •On average, all trash could be removed by two individuals within 10 to 20 minutes •Approximately 2-3 times more trash than the low condition category			•Predominately littered except for a few clean areas •Trash is evident upon first glance in moderate levels along site •Evidence of site being used by people: scattered cans, bottles, food wrappers, plastic bags, etc. •On average, would take a more organized effort (more than 2 people, but less than 5) to remove all trash from the area. Removal of trash would take 10 to 30 minutes •Approximately 2-6 times more trash than the moderate condition category			•Trash is continuously seen throughout the site •Trash distracts the eye on first glance •Substantial levels of litter and debris in streambed and banks •Evidence of site being used frequently by people (e.g., many cans, bottles, food wrappers, plastic bags, clothing, piles of garbage and debris) •On average, would take a large number of people (more than 5) during an organized effort to remove all trash from the area. Removal of all trash would take >40 minutes •Approximately >2 times more trash than the high condition category		
Site Score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

Figure 2. Trash category and scoring descriptions from Estuary Station Trash Condition Categories and Scoring System Form.

Figure 3. Example of “Low” Trash Condition Category.

Figure 4. Example of “Moderate” Trash Condition Category.

Figure 5. Example of "High" Trash Condition Category.

2. **Determine the Locations of Storm Drain Outfalls and Homeless Encampments within the Station**

Record the number and size of stormwater outfalls (>18 inches) in the sampling area and record this information in the **Stormwater Outfalls/Encampments** section on the **Estuary Station Trash Condition Categories and Scoring System Form** for each outfall present. Outfalls include any pipes or discharge areas from outside of the estuary. Outfall categories are as follows: 18–24 inches, 25–36 inches, 37–48 inches, and >48 inches. Record if there is trash at the outfalls and the amount of trash present. Trash at the outfalls is the trash that is in the immediate vicinity of, and appears to have come out of, the outfall. Trash count (number of items) categories are as follows: <10, <50, <100, and ≥100.

Determine whether there is a homeless encampment in the sampling area or within 200 meters of the sampling area, either upstream or downstream. Record this information in the **Stormwater Outfalls/Encampments** section on the **Estuary Station Trash Condition Categories and Scoring System Form**.

3. **Take Photographs of Station Area**

Photograph trash conditions by taking one photograph from each edge of the station area (the polygon) facing the center to document general trash conditions and any significant features that may influence the presence of trash (**Figure 6**). Additional photographs may be taken to document site conditions. Each Photograph ID should use the following naming convention: Unique SiteID-Station-Location-Survey Date (month.day.year), e.g., SC-NEW-EDGE1-STATION1-10.01.2023. Complete the Photo Documentation section of the **Estuary Station Trash Condition Categories and Scoring System Form** to record the metadata on the images taken (**Table 1**).

Photos should be uploaded to the appropriately named shared folder at the following link: Bight 23 – Trash Estuary Photos or emailed directly to leahth@sccwrp.org.

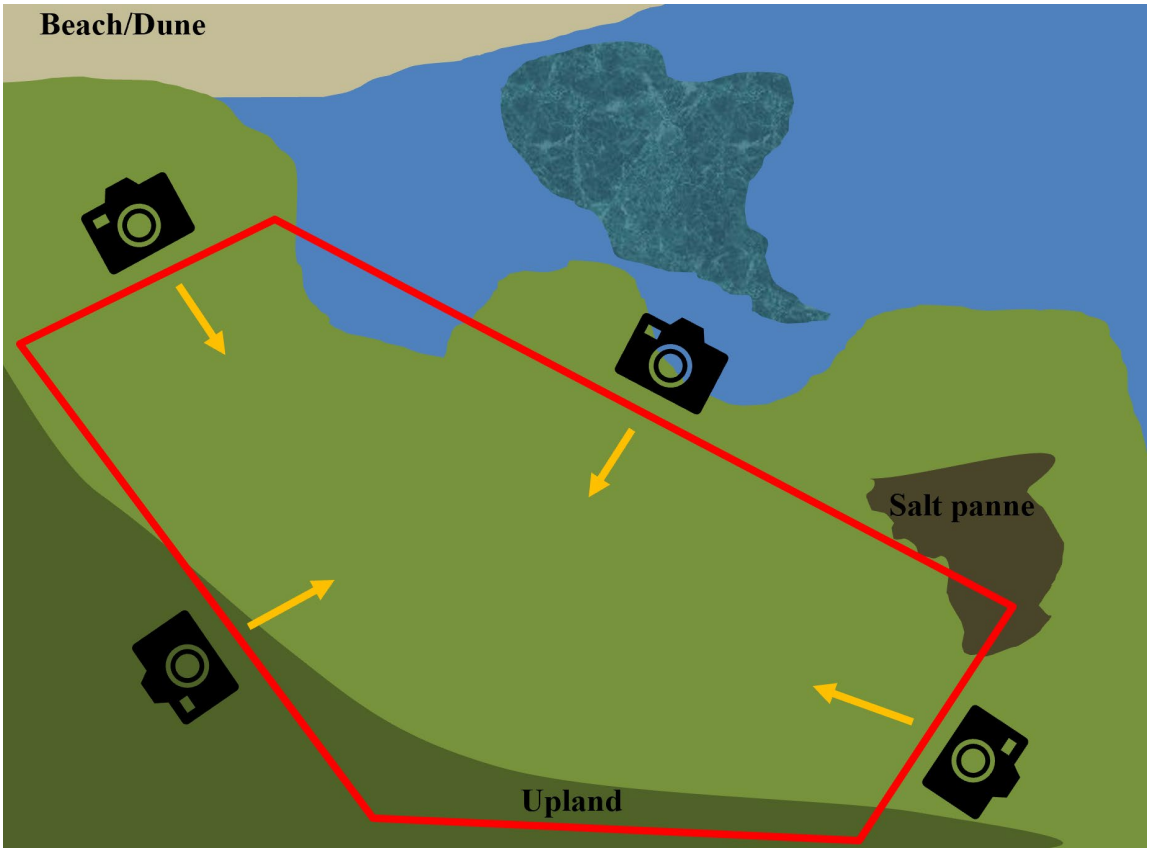


Figure 6. Representative diagram of photographs to be taken of the station sampling area.

Table 1. Photograph documentation form for the visual observation method.

Location	Photograph ID
Station Edge 1	
Station Edge 2	
Station Edge 3	
Station Edge 4	
Misc 1	
Misc 2	

4. TIME SEARCH: Record the Number of Each Trash Item Found

Within the station sampling area, select the area where the majority of trash items are found (the wrack line should be prioritized for sampling; if there is no wrack line, then target an area with high abundances of trash). This method should be completed by two people – one person calling out trash and one person recording it on the tally datasheet. The recorder should familiarize themselves with the datasheet prior to surveying.

Starting from one edge, tally each trash item walking slowly while visually scanning for trash. Scan an area within a shoulder-width zone to avoid missing small or partially covered items and continue the process for **10 minutes** or until all trash items have been tallied. A larger scan width may be used at sites with little or no vegetation. The recommended approach for field teams is to adopt a systematic scan approach while walking in the assessment area. An approach that focuses on walking a continuous S-shaped path over the sampling area, as shown by the example of the lawnmower pattern in **Figure 7**, provides a systematic approach for observing trash and minimizing opportunities for missing small or difficult-to-observe trash pieces.

Trash is divided into nine major categories, including plastic, miscellaneous, fabric and cloth, biodegradable, biohazard, construction, glass, large, and metal. From there each category is broken into different items. For example, the plastic category is broken down into Bag - reusable, Bag - recyclable, Bag pieces, Balloons, etc. Counts of each item within a category should be measured by tallying them on the **TIME SEARCH: Estuary Station Quantitative Tally Method Datasheet**. If multiple pieces of trash in the same approximate area appear to come from the same item (i.e., as broken-off fragments), count the pieces as one item; however, if the pieces appear to come from different items, count them separately. For example, if multiple pieces of plastic bags are present and they all are the same color and thickness, and all appear to be weathered similarly, count the item as one. Otherwise, the pieces should be counted separately.

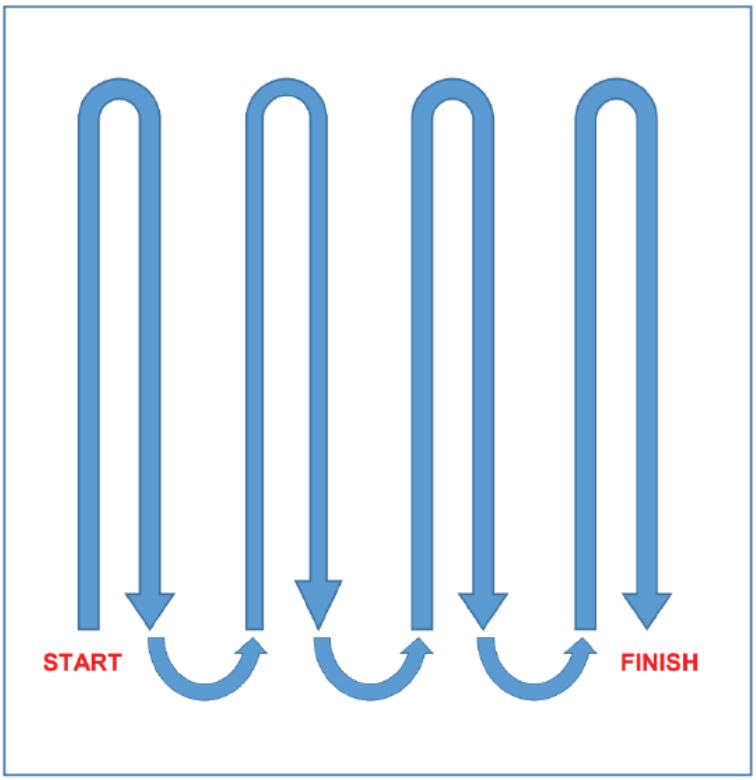


Figure 7. Example "Lawnmower Pattern" for systematically walking assessment area for estuary surveys.

The area where trash was assessed should also be estimated. The latitude and longitude should be recorded at the beginning and end of the scanned area. The approximate width of the area should also be estimated.

Estuary Quantitative Tally Method Datasheet

Circle one: **TIME SEARCH or Vegetation:** *Complete one data sheet per quadrat if trash is found*

Station ID: _____ Date: _____Initials: _____ Transect #: _____ Start time:_____ End time:_____

TIME SEARCH ONLY: Start Lat: _____ Long: _____ Finish Lat: _____ Long: _____ Approx. Width Scanned: _____Habitat type: _____

Plastic	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**	**Total**
Bag											
Balloon											
Bottle											
Cigarette											
Container											
Cups											
Foam*											
Lighters											
Pens/Markers											
Straw											
Wrapper*											
Plastic Other											
Fabric and Cloth											
Fabric/ Cloth											
Shoes											
Fabric Other											
Large											
Furniture/Appliances											
Shopping Carts											
Tires											
Large Other											
Biodegradable											
Paper/Cardboard											
Biodegradable Other											
Biohazard											
Dead Animals											
Human Waste/Diapers/TP/Condom											
Mask											
Medical waste/Latex gloves											
Pet Waste											
Biohazard Other											
Construction											
Materials - brick, concrete, wood											
Rebar											
Construction Other											
Glass											
Glass Bottles											
Glass Pieces*											
Glass Other											
Metal											
Cans											
Auto Parts											
Batteries											
Nails, Screws, Bolts, etc.											
Spray Paint Cans											
Wire (barb, chicken, etc.)											
Metal Other											
Miscellaneous											
Ceramic Pots/Shards											
E-waste											
Rubber/Rubber pieces											
Sports Balls											
Misc. Other											
TOTAL:											

***These items may be binned if abundance is greater than 10 pieces as follows:**

M= 11-100 pieces; H ≥ 101 pieces

Estuary Station Trash Condition Categories and Scoring Form

*Complete one data sheet per **station***

	Trash Condition Category											
	Low			**Moderate**			**High**			**Very High**		
Description	•Effectively no or very little trash •On first glance, little or no trash is visible •Little or no trash is evident when site is closely examined for litter and debris •One individual could easily remove all trash observed within 10 minutes			•Predominately free of trash except for a few littered areas •On first glance, trash is evident in low levels •After close inspection, small levels of trash are evident in site •On average, all trash could be removed by two individuals within 10 to 20 minutes •Approximately 2-3 times more trash than the low condition category			•Predominately littered except for a few clean areas •Trash is evident upon first glance in moderate levels along site •Evidence of site being used by people: scattered cans, bottles, food wrappers, plastic bags, etc. •On average, would take a more organized effort (more than 2 people, but less than 5) to remove all trash from the area. Removal of trash would take 10 to 30 minutes •Approximately 2-6 times more trash than the moderate condition category			•Trash is continuously seen throughout the site •Trash distracts the eye on first glance •Substantial levels of litter and debris in streambed and banks •Evidence of site being used frequently by people (e.g., many cans, bottles, food wrappers, plastic bags, clothing, piles of garbage and debris) •On average, would take a large number of people (more than 5) during an organized effort to remove all trash from the area. Removal of all trash would take >40 minutes •Approximately >2 times more trash than the high condition category		
Site Score	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**

Stormwater Outfalls/Encampments							
Number of stormwater outfalls in the site > 18" in diameter:							
18-24"	_____	25-36"	_____	37-48"	_____	>48"	_____
Trash at outfalls? Yes / No							
Amount of trash present in outfall (# of pieces):	<10	<50	<100	>100	(circle one)		
Homeless encampment within 200 meters of station? Yes / No					(circle one)		

Photo Documentation (if time)	
Location	**Photograph ID + Photographer initials**
Station Edge 1	
Station Edge 2	
Station Edge 3	
Station Edge 4	
Misc 1	
Misc 2	

Appendix C: Data Sheet for Epibenthic Marine Debris

BIGHT '23 TRAWL DEBRIS FORM **Agency:**_____ **Page**_____**of**_____

Station:_____ **Trawl #:**_____ **Date:**_____ ☐ **CHECK HERE IF NO DEBRIS PRESENT IN SAMPLE**

Anthropogenic Debris – include Brand names in Comments if known

Plastic	**Count**	**Comment**
Bag		
Bandaid		
Balloon (mylar/latex)/Ribbon		
Bottle		
Buoy		
Cap/Lid		
Cigarette Box/Wrapper		
Cup		
Filmstrip (movie)		
Fishing Line/Net		
Food Bag/Wrapper		
Polypropylene Rope		
Single use food container		
Toy		
Utensil		
Plastic Piece (unid.)		
Other Plastic (comment req.)		
Glass		
Beer Bottle		
Other Glass Bottle/Jar		
Glass Piece (unid.)		
Other Glass (comment req.)		

Misc. Items/Pieces	**Count**	**Comment**
Boat/Engine/Engine Part		
Clothing		
Concrete/Asphalt		
Fiberglass		
Food		
Latex/nitrile gloves		
Leather		
Lumber		
Mask–specify single use or cloth		
Paper		
Rag/Cloth		
Rubber		
Shoe		
Tape		
Tire		
Other misc. (comment req.)		
Metal		
Drink Can		
Can – other		
Fishing Gear		
Wire		
Metal piece (unid.)		
Other metal (comment req.)		

Natural Debris

Marine Origin	**Count**	**Est.***	**Comment**
Foliose Algae – not kelp			
Gorgonian Sea Fan (dead)			
Kelp Holdfast			
Kelp Stripe/Blade			
Other Foliose Algae			
Rock			
Seagrass			
Other Marine (comment req.)			

Terrestrial Vegetation	**Count**	**Est.***	**Comment**
Leaves/Seed Pod			
Stick/Branch/Driftwood			
Other Terrestrial (comment req.)			

***For Natural Debris only**, if the count >10 and an exact count cannot be made, leave the "Count" column blank and estimate the amount (M or H) in the "Est." column.

Moderate: **M = 11-100**

High: **H = >100**

Completed by: _____ (name, agency)

Appendix D: Microplastic Sample Collection and Analysis Protocols

Offshore Sediment Collection for Microplastics Analysis

Materials (Items indicated by an asterisk will be provided by SCCWRP in field sampling kits)

- Prepared water field blanks*
- Empty pre-kilned 16-ounce mason jars, pre-labelled*
- Metal spade
- Microplastics Analysis Grade (MAG) water*
- Pre-ashed heavy-duty aluminum foil*
- 500 mL laboratory-grade polypropylene squirt bottle*
- Cooler
- Bubble wrap*
- Field data sheets

Sample Collection

Atmospheric sample contamination is a concern when collecting environmental samples for microplastics analysis. While background contamination is often unavoidable, working quickly and frequently rinsing materials with MAG water will greatly help reduce excess contamination. Prepare as many materials as possible ahead of time and become familiar with all procedures in advance. ***Sediment for microplastics analysis will be collected first to reduce chances of background contamination.***

1. If there are large amounts of debris on the Van Veen, the sampler should be dunked or rinsed with ambient water.

2. Deploy the sediment sampler to collect sediment.

3. Upon retrieval of the sediment sampler, **open the previously prepared field blank jar(s), leaving the jar opening exposed as close as possible to where the sediment will be collected.** Wrap the lid with foil and set aside.

4. Thoroughly rinse the metal spade and an empty, pre-kilned 500 mL glass jar three times with MAG water using the squirt bottle.

5. Fill the empty, pre-kilned 16-ounce glass jar (~500 mL) from **the top 5 cm of sediment**. Rinse the jar lid and the piece of aluminum foil with MAG water. Cover the mouth of the jar with the piece of foil and screw on the lid tightly. If sediment is spilled on the outside of the jar, it can be cleaned with a gloved finger or cellulose wipe such as paper towel.

6. **Immediately** after the sediment sample has been collected, remove the field blank lid from the foil and rinse three times with MAG water. Cover the jar opening with the rinsed piece of foil and replace the lid.

7. Label both the sample jar and the field blank jar with the date and check that pre-labelled SiteID and Agency values are correct. Wrap both jars in bubble wrap and return to the cooler.

8. All samples should be stored at 4 °C and returned to SCCWRP.

Intertidal Wetland Sediment Collection for Microplastics Analysis

Materials (Items indicated by an asterisk will be provided by SCCWRP in field sampling kits)

- Prepared water field blanks*
- Empty pre-kilned 16-ounce mason jars, prelabelled*
- Aluminum corer – 5 cm diameter, 5 cm length*
- Metal spoon*
- Microplastics Analysis Grade (MAG) water*
- Aluminum foil*
- 500 mL laboratory-grade polypropylene squirt bottle*
- Cooler*
- Bubble wrap*
- Gloves*
- Field data sheets

Sample Collection

Atmospheric sample contamination is a concern in collecting environmental samples for microplastics analysis. While background contamination is often unavoidable, working quickly and frequently rinsing materials with MAG water will greatly help reduce excess contamination. Prepare as many materials as possible ahead of time and become familiar with all procedures in advance.

Within each estuary, one sediment sample will be collected from station #2. If the estuary has only one station, one sediment sample will be collected from station #1.

1. Select an undisturbed area in the intertidal zone approximately 5 x 5 ft within the station sampling area.

2. Put on gloves.

3. Rinse the aluminum corer thoroughly with MAG water.

4. Open the previously prepared field blank jar(s), leaving the jar opening exposed as close as possible to where the sediment will be collected. Wrap the lid with foil and set aside.

5. Push the aluminum corer into the sediment to a depth of 5 cm (to the top of the corer)

    a.**Do not touch the top of the core

6. Rinse the sample jar x3, lid, foil, and metal spoon with MAG water.

7. Extract the corer from the sediment. With the corer still inserted in the sediment, dig out the sediment from the side of the corer using a metal spade or by hand.

    a. Use care to not push the sediment out of the core when sliding your hand underneath the core.

8. Hold the corer over the sample jar in case the sediment falls out unexpectedly. Use your gloved hand or the previously rinsed metal spoon to push the sediment into the sample jar, if necessary. Cover the lid of the sample jar and the field blank jar with the previously rinsed foil. Re-rinse the tin foil before covering the jars.

9. Repeat steps 3-7 until 3 sediment cores have been collected. Each sediment sample will be deposited into the same sample jar. Cores should be taken ~1.5 ft apart within the sampling area where the sediment has not been previously disturbed (e.g., stepped on).

10. Label both the sample jar and the field blank jar with the date and check that pre-labelled SiteID and Agency values are correct. Wrap both jars in bubble wrap and return to the cooler.

11. All samples should be stored at 4 °C and returned to SCCWRP headquarters.

    a. Cores should not be frozen.

Shellfish Collection for Microplastics Analysis

Modified from SCCWRP internal SCCWRP shellfish collection, cleaning, and shucking protocols

Sample Collection

Materials (Items indicated by an asterisk will be provided by SCCWRP in field sampling kits)

- GPS
- Field data sheets
- Polyethylene or nitrile gloves
- Rubber mallet
- Chisel
- Pre-ashed heavy-duty aluminum foil*
- Measuring tape or ruler
- Wet ice
- Cooler

Shellfish are to be collected at low tide when shells are closed to facilitate access to shellfish beds and reduce potential microplastics background contamination.

1. Locate site (pre-selected by Bight '23 Shellfish Element) using GPS.

2. Locate shellfish within 200 meters of the target latitude. Shellfish may be embedded in the sediment or attached to hardscapes.

3. Collect shellfish. Oysters (*Crassostrea gigas*) of approximately 10 cm to 15 cm in length are recommended. Mussels (*Mytilus californianus* or *Mytilus galloprovincialis*) of approximately 55 mm to 65 mm in length are recommended.

> 3.1. Some shellfish 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.
>
> 3.2. If shellfish 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 oyster 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.*

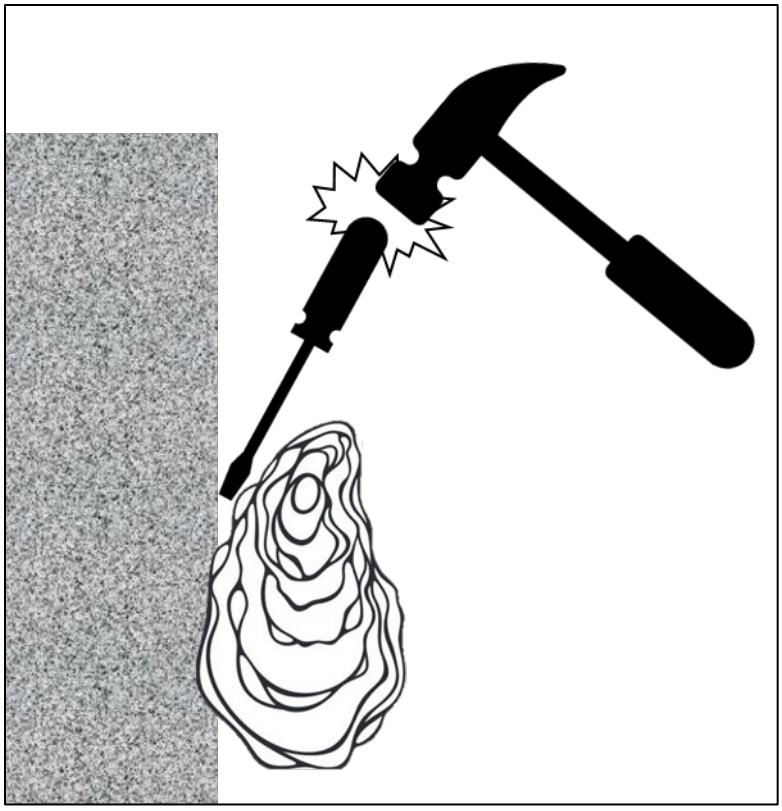


Figure 1. Removal of shellfish from hardscape using mallet and chisel.

4. Upon collection, rinse each shellfish in ambient water to remove excess debris and wrap each individual shellfish in a piece of pre-ashed heavy-duty aluminum foil. All shellfish designated for microplastics analysis should be placed in a separate cooler on ice. Keep the coolers in the shade. All samples should be stored on ice or at 4°C and returned to SCCWRP within 24 hours of collection.

Shellfish Cleaning and Shucking

Shellfish designated for microplastics analysis will be cleaned and shucked at SCCWRP. If not shucked fresh, shellfish will be preserved in 1 µm filtered 70% EtOH and stored at 4°C prior to shucking.

Materials

- Cotton lab coats
- Nitrile gloves
- 2 small buckets
- Natural fiber scrub brush
- Petri dish, kilned and rinsed with MAG water
- 20 µm filter paper
- Pre-ashed heavy-duty aluminum foil
- Microplastic analysis grade (MAG) water
- Heavy-duty garden gloves
- Shucking knife
- Calipers
- Scissors, rinsed with MAG water
- Balance
- Polypropylene sample jars, pre-weighed

Shellfish must be cleaned and shucked within 48 hours of collection and never frozen. Shellfish may be temporarily stored on ice or at 4°C. All shellfish designated for microplastics will be cleaned and shucked at SCCWRP to minimize possible background contamination.

All shellfish cleaning is completed in the biology laboratory using the stainless-steel sinks and spray hoses. Once shellfish are clean, shucking is completed in the chemistry laboratory in the clean cabinet. All personnel must wear cotton lab coats and nitrile gloves while cleaning and shucking.

1. In the biology lab, fill up the small bucket with tap water. Use the natural fiber scrub brush to clean mud/algae/debris off the exterior of the shellfish, paying special attention to where the two shells meet. Periodically dip the shellfish into the bucket and use the spray hose to wash off debris. Shellfish are clean when the water runs clear. Place clean shellfish in a second, clean bucket until at least enough shellfish for a replicate (5) have been cleaned and are ready for transfer to the chemistry lab for shucking.

2. Once the shellfish are clean, bring them into the chemistry lab.

3. Set up the balance and a dissection blank (i.e., open petri dish with wetted 20 µm filter paper) inside the clean cabinet. Lay a down a large piece of foil inside the clean inside the clean cabinet.

4. One at a time, rinse the outside of the shellfish thoroughly with MAG water over the sink.

5. Bring the shellfish to the clean cabinet and place it 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.

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

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

7. Once pierced, shove the blade of the shucking knife ~50-70% into the shellfish. Use this to pry open the oyster 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. Rinse the polypropylene sample jar three times with MAG water and record its mass. Place the empty sample jar on the balance, tare the balance, and use the shucking knife to slide the shellfish viscera into the jar. Record the mass. Close the jar.

Note: Shellfish tissue may be cut into smaller pieces after depositing into the sample jar with scissors to facilitate tissue digestion.

9. Repeat steps 4-8 until 5 shellfish have been deposited into the sample jar, taring the balance each time. Between each composite sample, replace foil and rinse the shucking knife and scissors with MAG water.

10. Store the samples in the walk-in freezer at -20°C in the turquoise bin labeled “Bight 23 Microplastics Mussels”.

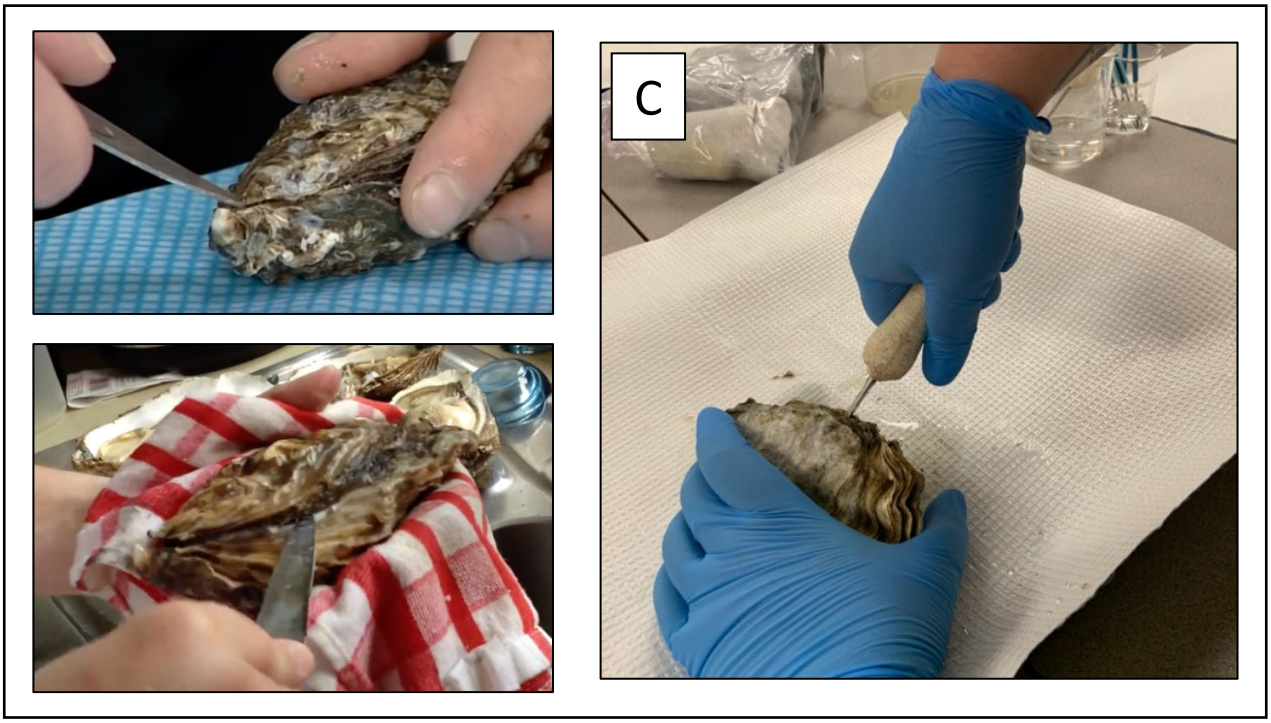


Figure 2. (A) Example of piercing from hinge. (B) Example of piercing from un-frilled side. (C) Example of how deep the shucking knife should be inserted into the oyster for shucking.

Microplastics Analysis for Sediment

The following protocol is adapted from Langknecht et al. (2023)

PURPOSE

This SOP describes the procedure by which microplastics >125 µm in longest length will be extracted from sediment samples, picked, quantified, characterized, and chemically identified. A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

Microplastic particles will be extracted from sediment using a density separation. An optional wet peroxidation step is also provided to destroy excess organic materials as needed. All suspected microplastic particles are enumerated, measured (i.e., length and width), and characterized by color and morphology. A minimum of 75 suspected microplastic particles are randomly subsampled across all size fractions for spectroscopy analysis to determine material type. Particles down to 125 µm will be analyzed.

Method extractions should be conducted in a laboratory environment that minimizes the use of plastics and prevents plastic contamination from airborne particles, ideally with HEPA filtration and positive air pressure. Laboratory conditions and procedures to mitigate background contamination will be captured during data submission (**Appendix E**). A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

MATERIALS

For extraction

Item	**Details**
Natural sponge	Amazon - “Natural Sea Sponge 6-7"
Heavy duty aluminum foil	Heavy duty aluminum foil is necessary if kilned at 500 °C.
Laboratory Labelling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
Squirt bottles (polypropylene)	Amazon – “Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle”
Metal spoon or spatula	-
Microplastics analysis grade (MAG) water	MilliQ (18 MW cm), or Deionized water filtered through a 1 µm pore-size filter (Suggestion: polycarbonate (PCTE) membrane filter from Sterlitech Catalog no. PCTF1047100). Do not use glass fiber filter.
1 µm pore-size filters	Material and diameter will vary based on and filtering apparatus; Suggestion: Sterlitech Catalog no. PCTF1047100
20 µm pore-size filters	Material and diameter will vary based on and filtering apparatus; Suggestion: Sterlitech Catalog no. 1270175
Metal sieves	Gilson Catalog no. V8SF 5M (5 mm mesh size) VWR Catalog no. 57334-568 (500 µm mesh size) VWR Catalog no. 57334-572 (355 µm mesh size)

	VWR Catalog no. 57334-584 (125 µm mesh size)
Metal sieve pan	Same diameter as sieves
NaBr or $CaCl_2$	ThermoFisher Scientific Catalog No. 212675000 VWR Catalog No. 97062-590
2 x 2L glass separatory funnels	VWR Catalog no. 30356-722 Note: 1L separatory funnels may be used, but 2L are generally preferred
1 x glass funnel	VWR Catalog no. 89090-626
50 mL conical polypropylene centrifuge tubes	Fisher Scientific Catalog NO. 14-432-22
30-35% Hydrogen peroxide	100 mL per round of wet peroxide reactions per sample
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858
2 x Glass Beakers, 2 L	VWR Catalog no. 10754-760
Magnetic Stir bar	Fisher Catalog no. 14-513-67
Weighing Balance	Satorius Item no. ENTRIS2201I-1SUS
Stir plate	Fisher Catalog no. S504631H
Drying oven (set to 45°C)	-
Surrogate	Polyethylene microspheres, 600-700 µm, blue color, (Cospheric, catalog no. BLPMS-1.00, 600-700 µm) Polyethylene microspheres, 300-355 µm, green color, (Cospheric, catalog no. GPMS-0.98 300-355 µm) Polyethylene terephthalate fibers, >500 µm, orange color

For counting

Item	**Suggested Materials**
Glass Petri Dishes for wet picking	VWR Catalog no. 25354-069
Small Glass Petri Dishes for dry picking from a filter	VWR Catalog no. 25354-025 (For use with a 47 mm diameter filter)
Petri Dishes for picked particles	Size and material not specified
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”

Laboratory labeling tape	-
Aluminum foil	-
Double sided tape	Available from stationary stores
Clear projector paper	Available from stationary stores
Metal teaspoon	Amazon - “4.5" Stainless Steel Teaspoon, Set of 6”
Stereoscope	Interchangeable black and white base preferable for picking
Microscope digital camera attachment	E.g. ToupTek touptek.com/product/product.php?lang=en&class2=56
Computer with software for images and measurements	E.g. - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Safety glasses, goggles, or face shield when applicable (e.g., when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)
- Functioning fume hood (when working with reagents)

PROCEDURE

Take notes on everything you do, especially any deviation from the wording of the SOP.

Procedural Blanks

- Run one laboratory blank with each set of test samples; the blank will consist of an empty 16 oz pre-kilned glass mason jar, identical to those used in your laboratory for the digestion, run through the same protocol as the test samples; extracted, size fractioned, particles quantified, characterized, and chemically identified.

A. Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with MAG water.
- Clean sieves with soap and water using a natural sponge, sequentially rinse with tap water, deionized water, and MAG water. Dry in a clean fume hood.
- When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Prepare NaBr or $CaCl_2$

1. Make a sodium bromide (NaBr) or calcium chloride ($CaCl_2$) solution with ϱ=1.4g/cm^3. Use a hydrometer to check density. Filter the solution through a 1 µm filter to remove any particulates. Recheck and record the density to make sure it hasn’t changed. If the density of the solution has changed after filtering, the density should be adjusted and refiltered before proceeding. Fill a laboratory wash bottle with 400 mL of this solution.

Sample Preparation

1. Homogenize the sample in jar by thoroughly mixing using a metal spoon for approximately 3 minutes

2. Subsample 1 g of wet sediment for the determination of moisture content. Record the initial mass of the sediment. Dry the sediment in an oven at 60°C for 24 hours. Remove the sediment from the oven. Place the sediment in a desiccator and allow it to cool completely. Re-weigh the sediment once it is completely cool. Alternatively, sediment may be freeze dried to remove moisture. Record the final mass of the sediment. Calculate the moisture content of the sediment according to the following equation where *w* is wet weight and *d* is dry weight.

$$Moisture\ Content\ (\%) = \frac{w - d}{w} \times 100$$

3. Subsample 100 g of wet sediment for extraction. If 100 g wet weight of sediment leads to difficulties during sample processing, a minimum of 50 g of wet sediment may be used for extraction. Record the mass of the wet sediment extracted.
4. Spike the surrogate MPs (10 particles per type representing the smallest and largest size fractions) onto the sediment. Under a microscope, pick and transfer particle onto the sediment with a fine tip tweezer. To avoid airborne particles contaminating the sample, keep the sieve covered with aluminum foil when not adding the particles to the sediment.
5. Stack sieves in the following order from top to bottom: 5 mm, 125 µm, and sieve pan. Pour the sample (100 g wet) over the stacked sieves and use MAG water to gently rinse smaller debris through sieves. If sediment is too dense to pass through the sieves, it may be mixed with MAG water in a beaker to loosen.
6. Rinse the original sample container (if subsampled sediment temporarily stored separately) and any tools used to help transfer materials (i.e., spatulas, glass jars) onto the sieve stack a minimum of 5 times. Continue rinsing the sample until water passing through the sieves runs clear. Rinse down the walls and sides of the sieves very carefully, a minimum of five times. *Note: It is critical to avoid losing any of the sample in this transfer step.*
7. Sediment collected on the 125 µm sieve will be retained. Discard materials collected on the 5mm sieve and sieve pan. *Note: Allow sediment in the sieve pan to settle. Pour out water before discarding sediment in the trash.* Your sample size fraction is now 125-5000 µm. Cover the sieve with aluminum foil.

B. Extraction Procedure

Phase I: Density Separation

The following method describes a density separation technique using a separatory funnel but other types of glassware (e.g., beakers) may be used in a similar capacity.

In lieu of a separatory funnel, a glass funnel with flexible tubing attached to the stem may be used. A clamp is used to close the tubing to prevent the sample from falling through. After each density separation, the sediment and supernatant are captured in two separate beakers by allowing the sample to flow through the tube by removing the clamp. The funnel is then rinsed thoroughly with NaBr or $CaCl_2$ into the beaker with the supernatant to capture any particles that may have adhered to the side of the funnel. This process is repeated with each density separation.

1. Make sure that the separatory funnels' stoppers are in the "closed" position before beginning. Hold and tilt the 125 µm sieve. Rinse the sieve contents with MAG water towards the bottom of the sieve so that all particles are in one concentrated area. Rinse the sieve contents with NaBr or $CaCl_2$ solution to remove the MAG water in the sediment. Now, use the 400 mL (1.4 g/cm^3) NaBr or $CaCl_2$ solution to rinse remaining debris into a 2-L glass separatory funnel, using a glass funnel if necessary. A metal spatula may be used to gently scrape contents of the 125 µm sieve into the separatory funnel if necessary. *Note: The only liquid entering the separatory funnel should be NaBr or $CaCl_2$ solution*.

2. Rinse down the tilted sieve into the separatory funnel with the NaBr or $CaCl_2$ solution a minimum of five times to ensure complete transfer of all debris into the separatory funnel. Thoroughly rinse the sieve, spatula, and glass funnel into the separatory funnel with NaBr or $CaCl_2$ solution so that all particles enter the separatory funnel.
3. Pour the rest of the allotted 400 mL NaBr or $CaCl_2$ solution into the separatory funnel, retaining a small amount of NaBr (20-100 mL), so that the total amount of solution in the separatory funnel reaches ~300-380 mL.
4. Stopper the separatory funnel and tilt to a 90° angle. Shake vigorously for three minutes, ensuring NaBr $CaCl_2$ solution comes into complete contact with entire sediment sample.
5. Place separatory funnel upright in ring stand and use the remaining 20-100 mL NaBr or $CaCl_2$ solution to rinse the inside of the stopper and the inner walls of the separatory funnel. The goal is to remove any debris stuck to the inner wall of the separatory funnel, so they are in the NaBr $CaCl_2$ solution. Do not cap the separatory funnel with the stopper, cover the top with a piece aluminum foil instead.
6. Let the contents of the separatory funnel settle for two hours, or longer, until the sediment and debris are settled at the bottom of the separatory funnel. The water column should be mostly clear, with the exception of floating debris at the solution surface.
7. Remove the aluminum foil and carefully pour the supernatant from the top of the separatory funnel into a beaker (e.g., 2 L) or onto a sieve stack.
8. Perform the second round of density separation.
    a) Add another aliquot of NaBr $CaCl_2$ solution (~200-280 mL) into the separatory funnel. Repeat steps 4 to 7.
    b) Combine the supernatant by repeating step 7.
9. Perform the third round of density separation.
    a) Add another aliquot of NaBr $CaCl_2$ solution into the separatory funnel and let it settle down overnight.
    b) Combine the supernatant by repeating step 7.
10. Drain or wash off the sediment from the separatory funnel. Discard the sediment.
11. Sieving the supernatant. Stack sieves in the following order from top to bottom: 500 µm, 355 µm, 125 µm. Transfer the supernatant to the sieve stack. Rinse the sieve stack with MAG water. Rinse the sieve contents into labeled beakers.
    a) NaBr should be properly disposed of in accordance with State/Federal regulations.
12. Particles may be counted via wet or dry sorting. If wet sorting is desired and oxidation is not required to remove excess organic matter, proceed to Section C: Microscopy

Filtration

1. Place a clean 20 µm polycarbonate track-etched (PCTE) filter on a non-fritted glass filter base with a stainless-steel support screen attached to a 1-L vacuum flask. Turn on the vacuum and slowly pour the beaker contents onto the filter.
2. Some plastics may adhere to the walls of the vacuum apparatus; it is advised to rinse down the sides of the apparatus as thoroughly as possible before removing the filter.
3. Turn off the vacuum pump and remove the filtering funnel. *Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.*
4. Turn on the vacuum pump and carefully use MAG water to rinse any particles stuck to the base of the filtering funnel onto the 20µm filter.
5. Turn off the vacuum and carefully transfer the filter to a clean petri dish and cover. Avoid losing any particles from the filter while transferring.
6. Repeat steps 1-5 for all size fractions.

Optional: Oxidation

Prepare digestion solution

1. Place stir bar in a 1 L beaker, cover with aluminum foil and place on stir plate.
2. Using the metal spatula, weigh 7.5 g of FeSO4·7H2O into weigh boat and add to the 1 L beaker.
3. Add 500 mL of MAG water to the 1 L beaker.
4. Add 3 mL of concentrated sulfuric acid to the 1 L beaker. This can be done by pouring a small amount of sulfuric acid into a small beaker and pipetting 3 mL into the 1 L mixing beaker.
5. Turn on stir plate and mix until all particulate matter has dissolved.
6. Filter the solution before use. For filtering procedure set up vacuum filtration system (see filtering SOP) and use a 1 µm PCTE filter.
7. Store filtered digestion solution in a clean amber bottle for later use.

Sample digestion

1. Samples containing high concentrations of organic matter may need an oxidation step. This step may be performed on the supernatant immediately following size fractionation (step 11) or after vacuum filtration.
2. Prepare ice bath and set up a hot plate in the fume hood.
3. Thoroughly rinse the contents of each sieve or filter with MAG water into separate 500mL beakers (i.e., one beaker per size fraction).
4. Using a graduated cylinder, add 20 mL of Fe_2SO_4 solution to each beaker.
5. Using the graduated cylinder, add 20 mL of H_2O_2 to each beaker.
6. Cover the beakers using aluminum foil or a watch glass as the reaction progresses.
7. Monitor the temperature of the reaction using a thermometer. If the reaction appears slow (e.g., no bubbles forming and minimal color change), place the beaker on the hot plate, heating gently to initiate the reaction. When the temperature reaches 40°C, remove from hot plate. *Caution: do not use a stir bar here as it will heat rapidly and can cause steep spikes in temperature.*
8. Ensure the temperature of the sample does not spike or reach above 55 ºC. Temperatures in excess of 60 ºC begin to melt some plastics. If the temperature is rising quickly (>45°C) place sample in ice bath to reduce temperature. Once the temperature has settled at around 40 °C, remove from the ice bath and continue to monitor the temperature. Repeat this process, if necessary, to maintain a temperature <55 °C throughout the digestion.
9. Once the reaction appears to slow (i.e., no bubbles and minimal color change), wait until the sample settles back to room temperature (<30°C) before adding another 20 mL of H2O2. Place beakers in the ice bath to cool the contents if necessary.
10. Gently rinse built-up material on the sides of the beaker with MAG water between H2O2 additions, minimizing water use to limit dilution of the solution.
11. Repeat steps 5-10 until there is no reaction (i.e., bubbles or color change) or a ratio of 1:5 ratio Fe2SO4:H2O2 is achieved. Allow the solution to settle back to room temperature (30°C).
12. Pour the contents of each beaker through the appropriate sieve (i.e., 125 µm, 355 µm and 500 µm). For each beaker, rinse the aluminum foil/watch glass once and the beaker three times into the sieve.
13. Repeat filtration steps 1-5 before proceeding to microscopy.

C. Microscopy

This procedure generates three filters per sample (3 size fractions), which will allow isolated plastics particles to be analyzed by stereomicroscopy, Raman spectroscopy, and/or other types of identification procedures. A total of at least 75 suspected microplastic particles across all size fractions will be randomly or haphazardly subsampled during microscopy and analyzed via spectroscopy.

1. Bring all three size fractions over to the microscope (i.e., 125-355 µm, 355-500 µm, 500-5000 µm).
2. Using a systematic method of your choice, count all particles for each size fraction and record the color and morphology of each. Measure all particles subsampled for spectroscopy along the longest perpendicular axes (length and width). (This measurement can be done with the selected particles for instrumental identification using FTIR or Raman). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement (e.g., measure three lengths and one width for a fragment that has fractured along its length). Make note of the method used for measurement in this case.
3. For instrumental identification, two options can be adopted to manipulate the particles. One is keeping the particles on the filter intact and using the filter directly under instrument for spectroscopic measurement. Another is manually picked and transferred particles from the filter (or beaker for wet sorting) to a glass slide. *Note: This option is only doable for larger particles.*
    i. Randomly pick (subsample) at least 75 particles identified from the entire sample across all size fractions. To avoid bias when selecting particles for spectroscopic analysis, it is suggested that all particles are assigned unique identification numbers and a random number generator (e.g., randomizer.org) is used to determine which particles are selected for chemical identification and measurements (De Frond et al., 2023).
    ii. Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using (e.g., double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy). *This is a suggestion only; please store particles as you see fit, and record method used.* When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If fewer than 75 particles are identified in the entire sample, include all particles for spectroscopic analysis. Once particles have been subsampled, proceed to spectroscopic analysis.

Microplastics Analysis for Shellfish

The following protocol is adapted from Thornton Hampton et al., 2023

PURPOSE

This SOP describes the procedure by which microplastics >125 µm in longest length will be extracted from shellfish samples, picked, quantified, characterized, and chemically identified. A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

OVERVIEW

Microplastic particles will be extracted from tissues using potassium hydroxide (KOH). All suspected microplastic particles are enumerated, measured (i.e., length and width), and characterized by color and morphology. A minimum of 75 suspected microplastic particles are randomly subsampled across all size fractions for spectroscopy analysis to determine material type. Particles down to 125 µm will be analyzed. Particles less than 125 µm will be saved for future potential analysis.

Method extractions should be conducted in a laboratory environment that minimizes the use of plastics and prevents plastic contamination from airborne particles, ideally with HEPA filtration and positive air pressure. Laboratory conditions and procedures to mitigate background contamination will be captured during data submission (**Appendix E**). A laboratory blank will be run in addition to each set of test samples, used to monitor particles introduced via procedural contamination.

MATERIALS

For extraction

Item	**Details**
Natural sponge	Amazon - “Natural Sea Sponge 6-7"
Aluminum foil	Heavy-duty Al foil is necessary if pre-cleaning by ashing at 500 °C is done.
Laboratory Labelling tape	Fisher Catalog No. 15901A
Fine-tip sharpie	Sold at stationary stores
Squirt bottles (polypropylene)	Amazon – “Highfive 250cc Scientific Safety Wash Bottle Narrow Mouth Polypropylene/Plastic Squeeze Bottle Medical Label Tattoo Wash Bottle”
Microplastics Analysis Grade (MAG) water	Alternatives include MilliQ (18 MW cm), Deionized water or water filtered through a 1 µm pore-size filter
1 µm pore-size filters	Material and diameter will vary based on and filtering apparatus; Suggestion: Sterlitech Catalog no. PCTF1047100
10 µm pore-size filters	Material and diameter will vary based on and filtering apparatus; Suggestion: Sterlitech Catalog no. PCTF10047100
20 µm pore-size filters	Material and diameter will vary based on and filtering apparatus; Suggestion: Sterlitech Catalog no. 1270175
Metal sieves	Gilson Catalog no. V8SF 5M (5 mm mesh size) VWR Catalog no. 57334-568 (500 µm mesh size)

	VWR Catalog no. 57334-572 (355 µm mesh size) VWR Catalog no. 57334-584 (125 µm mesh size)
Metal sieve pan	Same diameter as sieves
Glass mason jars	>500 mL size One for each size fraction that will be wet picked Non-plastic lids preferred
Vacuum filtration system: 1 x Vacuum pump 2 x Plastic tubing 2 x 1000 mL Glass filtering flasks with rubber stopper 1 x filtering funnel 1 x filter holder with glass support 1 x metal clamp	GAST model DOA-P704-AA Tygon S3™ Laboratory Tubing Filtration set-up VWR Catalog no. 89428-970 Secondary filtering flask VWR Catalog no. 10545-858
Polypropylene sample jars	500 mL capacity One per sample VWR Catalog no. 30617-164
2 x Glass Beakers, 2 L	VWR Catalog no. 10754-760
Magnetic Stir bar	Fisher Catalog no. 14-513-67
KOH pellets	CAS 1310-58-3 Fisher Catalog no. P250-500
Weighing Balance	Satorius Item no. ENTRIS2201I-1SUS
Stir plate	Fisher Catalog no. S504631H
Drying oven (set to 45°C)	-
Liqui-Nox liquid detergent	Alconox Catalog no. 1232-1
Surrogate	Polyethylene microspheres, 600-700 µm, blue color, (Cospheric, catalog no. BLPMS-1.00, 600-700 µm). Polyethylene microspheres, 300-355 µm, green color, (Cospheric, catalog no. GPMS-0.98 300-355um). Polyethylene terephthalate fibers, >500 µm, orange color

For counting

Item	**Suggested Materials**
Glass Petri Dishes for wet picking	VWR Catalog no. 25354-069
Small Glass Petri Dishes for dry picking from a filter	VWR Catalog no. 25354-025 (For use with a 47 mm diameter filter)
Petri Dishes for picked particles	Size and material not specified
Superfine-tip forceps	VWR Catalog no. 63042-688
Petri dish grid stickers	Amazon - “Diversified Biotech PetriStickers PSTK-1070 Square Grid Label for Petri Dish, 70 Square Grid (Pack of 36)”

Laboratory labeling tape	-
Aluminum foil	-
Double sided tape	Available from stationary stores
Clear projector paper	Available from stationary stores
Stereoscope	Interchangeable black and white base preferable for picking
Microscope digital camera attachment	E.g. ToupTek touptek.com/product/product.php?lang=en&class2=56
Computer with software for images and measurements	E.g. - ImageJ imagej.nih.gov/ij/ (free to download) - ToupView touptek.com/product/product.php?lang=en&class2=74

Personal Protective Equipment (PPE)

The following PPE are mandatory for sample processing:

- Clean cotton lab coat
- Clean nitrile gloves
- Safety glasses, goggles, or face shield when applicable (e.g., when working with reagents)
- Clean cabinet or covered enclosure to reduce contamination (if available)
- Functioning fume hood (when working with reagents)

PROCEDURE

Take notes on everything you do, especially any deviation from the wording of the SOP.

Procedural Blanks

- Run one laboratory blank with each set of test samples; the blank will consist of an empty 500 mL polypropylene jar, identical to those used in your laboratory for the digestion, run through the same protocol as the test samples; extracted, size fractioned, particles quantified, characterized, and chemically identified.

A. Preparation

- Before using any glassware or tools, wash with soap and water (surfactant helps to remove contaminant microplastics). Rinse three times with tap water, then three times with filtered/MAG water.
- Clean sieves with soap and water using a natural sponge.
- When equipment/tools/labware are not being used, or when samples are not being analyzed, keep covered to prevent procedural contamination.

Prepare KOH solution (200 g/L)

Potassium hydroxide (KOH) is a caustic and irritant solvent. All researchers must use KOH in a ventilated fume hood, and wear laboratory gloves and eye protection at all times.

1. Clean 2 L beaker and stir bar. Place stir bar in 2 L beaker, cover with aluminum foil and place beaker on stir plate.
2. For the digestion you will require a volume of KOH solution approximately three times that of the sample volume. To make 1 L of 20% KOH solution, weigh 200 g KOH pellets and add to beaker.
3. Add 1 L of MAG water to the beaker to create a solution of 200 g/L.
4. Re-cover with aluminum foil and mix on stir plate until KOH is fully dissolved.

5. Once dissolved, allow the solution to return to room temperature before filtering.
6. Filter the solution. Set up vacuum filtration system (see filtering SOP) using a 1 µm PCTE filter.
   Note: Glass fiber filter should be avoided due to shedding fibers.
7. Store filtered KOH solution in a clean, labeled, polypropylene jar for later use (KOH etches glass).

B. Extraction Procedure: KOH Digestion

1. Label clean 500 mL polypropylene sample jars with lids, 1 per sample.
   Note: If samples have already been distributed into polypropylene sample jars and the volume is adequate for digestion, proceed to step 4.
2. Place each sample of shellfish tissue in a separate sample jar.
3. Triple rinse the fish tissue container (used for shipping) into the polypropylene sample jar, using 20% KOH.
4. Surrogate spiking. Spike the surrogate MPs (10 particles per type representing the smallest and largest size fractions) onto the tissue. Under a microscope, pick and transfer particles into the sample with a fine tip tweezer. To avoid airborne particles contaminating the sample, keep the polypropylene sample jar covered with aluminum foil when not adding the particles.
5. Add 20% KOH solution to the polypropylene sample jar so that the volume of the liquid is roughly three times that of the sample (minimum 100 mL).
6. Cap sample jars loosely and place in a temperature-controlled oven or water bath at 50°C for 48 hours to digest. If the sample is not completely digested after 48 hours due to large chunks of tissue remaining, continue the digestion to 72 hours.

C. Sieving and filtration

1. Set up sieve stack (from top to bottom; 5 mm, 500 µm, 355 µm, 125 µm, and sieve pan).
   a. *Note: An additional sieve with a pore size <125 µm may be added between the 125 µm sieve and pan if desired if clogging issues are anticipated with sieving or filtering.*
2. Warming the MAG water (max. 50 °C) will help dissolve fatty residues.
   a. *Note: Sieve the sample as soon as possible after removal from the oven. As it cools the sample will begin to solidify which is problematic for sieving.*
3. Remove the digested sample from the oven and pour the contents of the jar into the sieve stack. Rinse the sample jar and the lid with warm MAG water (~50 °C) and pour the MAG water onto the sieve stack and repeat at least three times.
4. Inspect the sample for fatty residues. If lipid residue is observed in the jar and on the lid at the discretion of the analyst, rinse with warm (~50 °C) detergent water and pour onto the sieve stack.
   a. Create a 1% solution of Liqui-Nox in MAG water. Filter the solution through a 1 µm PCTE filter. Set up vacuum filtration system (see filtering SOP) using a 1 µm PCTE filter.
   b. *Note: Glass fiber filter should be avoided due to shedding fibers.*
   c. *Note: Sieve the sample as soon as possible after removal from the oven. As it cools the sample will begin to solidify which is problematic for sieving. If necessary, keep the sample warm in a hot water bath before sieving. Keep several MAG squirt bottles in a hot water bath to keep warm during the rinsing process.*
5. Rinse the contents of each size fraction into a separate clean, labelled glass beaker using warm MAG water.
6. Set up vacuum filtration system with glass or stainless-steel filtration parts. NOTE: During vacuum filtration, you may wish to heat the sample in a hot water bath to no more than 55 °C and monitor the temperature with a thermometer. Add a small amount of sample to the filtering cup at a time. This will help the samples filter a bit

faster. If you fill the whole funnel at once, the water cools down during the time it takes to filter and slows down the process.

a) Assemble the filtering system and turn on the vacuum pump to drain excess water from the glass filter holder.
b) Turn off the vacuum pump.
c) Place a 20 µm PCTE filter onto the glass filter holder.
d) Place the filtering funnel on top of the filter and secure it with a clamp.

7. Turn on the vacuum pump and pour the sieve pan contents (<125 µm size fraction) through the filtration system.
8. Rinse the beaker and the sides of the filtering funnel three times with warm MAG water.
9. Turn off the vacuum pump and remove the filtering funnel.
   Note: Tweezers may be used to ensure the filter is not removed with the filtering funnel as you do this.
10. Turn on the vacuum pump and carefully use warm MAG water to rinse any particles stuck to the base of the filtering funnel onto the 20 µm filter.
11. Turn off the vacuum pump and carefully slide the 20 µm filter off the glass filter holder and place it in a labeled clean petri dish.
12. Repeat steps 3-8 to filter remaining size fractions (i.e., <125 µm, 125-355 µm, 355-500 µm, >500 µm) onto 20 µm filters. There will be a total of 4 filters after completely filtering the sample (<125 µm, 125-355 µm, 355-500 µm, >500 µm) unless additional filters are needed due to clogging. The <125 µm filter(s) should be stored in a glass petri dish for potential future analysis.

D. Microscopy

This procedure generates three filters per sample (3 size fractions, 125-355 µm, 355-500 µm, >500 µm), which will allow isolated plastics particles to be analyzed by stereomicroscopy, Raman spectroscopy, and/or other types of identification procedures. A total of at least 75 suspected microplastic particles across all size fractions will be randomly subsampled during microscopy and analyzed via spectroscopy. Bring all three size fractions over to the microscope (i.e., 125-355 µm, 355-500 µm, 500-5000 µm).

1. Bring all three size fractions over to the microscope (i.e., 125-355 µm, 355-500 µm, 500-5000 µm).
2. Using a systematic method of your choice, count all particles for each size fraction and record the color and morphology of each. Measure all particles subsampled for spectroscopy along the longest perpendicular axes (length and width). (This measurement can be done with the selected particles for instrumental identification using FTIR or Raman). For fibers, do not measure frayed projections and use segmented/curved lines where necessary. If a particle has broken apart, use your best judgement (e.g., measure three lengths and one width for a fragment that has fractured along its length). Make note of the method used for measurement in this case.
3. For instrumental identification, two options can be adopted to manipulate the particles. One is keeping the particles on the filter intact and using the filter directly under instrument for spectroscopic measurement. Another is manually picked and transferred particles from the filter (or beaker for wet sorting) to a glass slide. *Note: This option is only doable for larger particles.*
   i. Randomly pick (subsample) at least 75 particles identified from the entire sample across all size fractions. To avoid bias when selecting particles for spectroscopic analysis, it is suggested that all particles are assigned unique identification numbers and a random number generator (e.g., randomizer.org) is used to determine which particles are selected for chemical identification and measurements (De Frond et al., 2023).

Store the subsampled particles on a substrate relevant to the method of chemical identification you will be using (e.g., double-sided tape for particles that will be analyzed via Raman or benchtop ATR-FTIR, a reflective surface for reflectance FTIR spectroscopy). *This is a suggestion only; please store particles as you see fit, and record method used.* When using double-sided tape, the tape should not be laid directly into the base of a petri dish. Instead, we recommend using projector paper or a glass slide as a base to lay the tape on. The choice between the two may depend on the stage of the instrumentation you are using for chemical analyses. When using ATR-FTIR spectroscopy, double sided tape should only be used for larger size fractions to avoid the crystal coming into contact with the tape. If fewer than 75 particles are identified in the entire sample, include all particles for spectroscopic analysis. Once particles have been subsampled, proceed to spectroscopic analysis.

Spectroscopic Microplastics Analysis for Sediment and Shellfish

SOP for Microplastic Chemical Analysis Using FTIR spectroscopy (ThermoFisher Nicolet iN10)

PURPOSE

This SOP describes the procedure by which extracted microplastics >125 µm in size can be chemically identified using Fourier-transform infrared spectroscopy (FTIR) spectroscopy. This document is intended to provide basic guidance on operating this instrument, specifically as a refresher for those who have participated in the SCCWRP instrumental training course in November 2019. It is not intended to be a comprehensive reference.

OVERVIEW

The ThermoFisher Nicolet iN10 MX infrared microscope allows the user to rapidly acquire microscopic images and simultaneously collect infrared spectra of solid specimens through both point-based analysis and comprehensive spectral mapping. Here, the whole sample will be analyzed with little sample preparation. Particles will either be sorted and mounted on double-sided sticky tape in a petri dish or dispersed across a filter membrane after vacuum filtration. Each lab is expected to identify the polymer type of each particle where possible and report the quantity of particles of each polymer type using FTIR spectroscopy.

MATERIALS

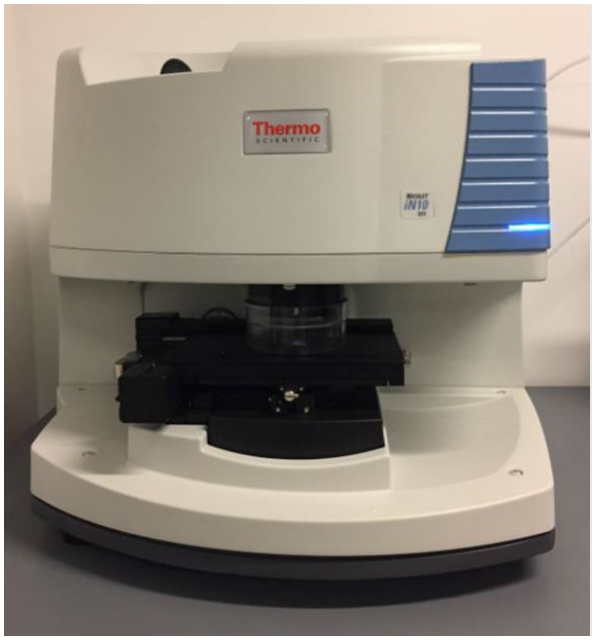


Figure 1. The ThermoFisher Nicolet iN10 MX infrared imaging microscope

Personal Protective Equipment (PPE)

The following PPE are mandatory for all stages of sample measurement:

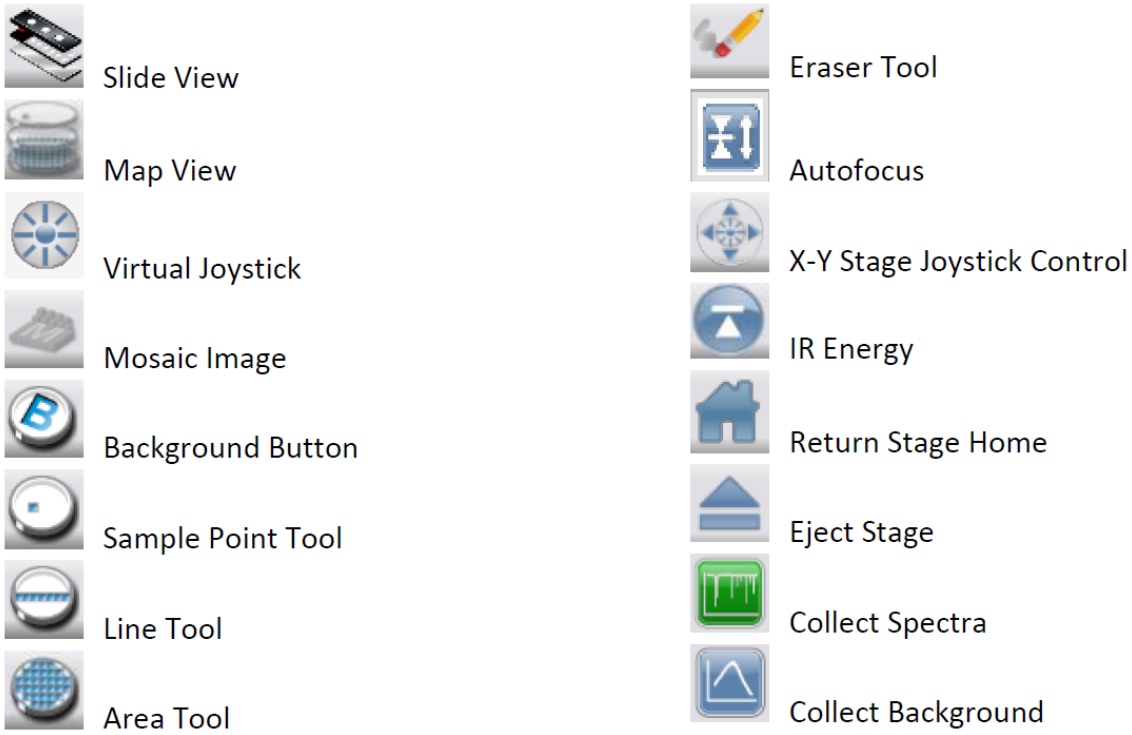
- Clean cotton lab coat
- Clean nitrile gloves

Safety and Precautions:

- Inspect the stage before moving it. Ensure that the stage, the ATR attachment, and your sample will not crash into the objective.
- Do not allow loose sample or particles to fall into the space under the stage.

- Follow the proper procedure utilized to cool the detectors.
- Do not move the stage when the ATR tip is in contact with a sample.
- Do not touch the tip of the ATR crystal with bare hands as it will transfer finger oils. Do not twist or turn the metal plate of the ATR crystal, it will take it out of alignment.

Picta Buttons:



A. Preparation - Fill the Liquid Nitrogen Dewars

It is important to follow the cooling process listed below, or the detectors and/or regions around them can get damaged. Follow all PPE requirements for handling liquid nitrogen (LN_2).

1. Preliminary Cooling of the Detectors – Add two funnels worth of liquid LN_2 to each detector.
2. Allow the Detectors to Cool – Wait 3 minutes for the detectors to cool.
3. Fill the Detectors with LN_2 – Fill both detectors (roughly 700 mL / fill the funnel 10 times). Do not allow excessive LN_2 overflow.
4. Allow Further Cooling – Wait 20 minutes before operating the instrument.

B. Sample Placement and Visualization

1. Open Picta software – Picta controls the microscope's imaging and FTIR capabilities.

2. Select a collection mode from the “View and Collect” tab.
   a. Transmission – For transparent or translucent samples, or samples on a salt window. Window MUST be IR transparent
   b. Reflection – For solid, opaque samples, surface analysis, and for use with particle wizard.
   c. ATR (Attenuated Total Reflectance) – For samples requiring contact-based spectra. Requires an additional attachment to the micro-ATR, equipped with a germanium crystal.
3. Select an IR Detector – There are three detectors with varying acquisition speeds and capabilities.
   a. Room Temperature – For general analysis of samples from 4000-400 cm^{-1}. Better to use for particles of ≥50 µm in size. Lower sensitivity and slower.
   b. Cooled Detector – Facilitates point, line, and area analysis of samples from 4000-675 cm^{-1}. Detects less noise than the room temperature detector. High sensitivity use for small particles.
   c. Imaging Detector – For rapidly acquiring line and area scans which span large areas. Does not facilitate point scans. Detection range from 4000-715 cm^{-1}.
4. Select a Resolution – Select a spectral resolution, resolution must be set based on the library selection for identity (i.e., if the library spectra are collected at 8 cm^{-1}, you must use that resolution to use that library). Select Normal (8 cm^{-1}) or high (4 cm^{-1}). Higher resolution takes a longer time.
5. Select Number of Background Scans – The background is a coded from multiple scans. Same number as sample scan or higher.
6. Select Background Frequency and Type – Backgrounds can be taken before or after analysis of each sample or at regular time intervals (every 300 minutes).
7. Select a Spectra Format – The data can be formatted into multiple types, for microplastics analysis the most common formats are transmittance or absorbance.
8. Enter Aperture Size –The default aperture setting is 150 x 150 µm. Aperture size is selected based on the size of the particle. Ensure the aperture window encloses the particle of interest only, without background.
9. Enter Number of Points – The number of points can be tailored for area and line scans. Increasing the number of points will cause the instrument to collect more spectra from more locations across your sample.
10. Record all above settings used.

C. Load and Locate Your Sample

1. Inspect the Stage – Check to make sure the stage and objective are free of obstructions.
2. Eject the Stage – Press the “Eject” button to make the stage more accessible.

 Eject

3. Insert your Sample – A sample can be placed on a microscope slide, the 3-hole slide, or the 12-spot slide. Clip the slide onto the stage to prevent movement.
4. Locate and Focus your Sample – Use the joystick, virtual joystick, autofocus, or the keyboard arrow keys to move the stage into position and focus on your sample.

 Autofocus  Virtual button

D. Capture an Image of your Sample

1. Select an Image Type – Press the “Map View” button. The detector can collect point, line, and area images.

2. Select an Area – Use the scroll button to determine the size of the area being analyzed. Draw a box, line, or point where you would like to collect an image.
3. Collect an Image – Press the “Capture Mosaic” button to collect an image. Right click to remove the area map ‘delete area map.
4. Save image – right click on the mosaic image and click save mosaic.

 Map View;  Point Tool;  Line Tool;  Area Tool;  Capture Mosaic

E. Collect a Background Spectrum

1. To Automatically Collect a Background – Select “Collect Backgrounds at Reference Location”. The sample holder has preset positions for collecting background spectra. The gold disk is used for reflection mode and the open hole is used for transmission mode.
2. To Manually Collect a Background – Move your sample to an area of interest, focus the image, press the “IR Energy” button and then the “Background” button. When using ATR mode, insert the ATR attachment and choose manual background collection. The background should be taken of the crystal itself.
3. Collect the Background – After a location has been marked, press the “Collect Background” button.

 IR Energy;  Background;  Collect Background

F. Collect a Spectrum

1. Focus the Sample – Use the joystick or virtual joystick to bring the image into focus.
2. Select a Spectra Tool – Either a point, line, or area scan can be produced.
3. Select a Spectra Type – Either a single spectrum or a map can be produced by selecting from the options in the bottom right-hand corner.
4. Ultra-Fast Mapping – Press “Ultra-Fast Mapping” for a fast scan with increased noise.
5. Collect Spectra – When in reflection mode - Optimize the “IR Energy” with the IR energy tool then press “Collect Spectra” button in the bottom-right corner. When in ATR mode - press “Collect Spectra” button in the bottom-right corner.
6. To save the raw spectrum – Click “File” >> “Save As” >> Name your spectrum. Save as a ‘.SPA’ file.

 Collect Spectra

G. Data Analysis and Library Searching

1. Set up a Library – In the “Analyze Spectra and Maps” tab scroll down to “Library Set-Up” and “Select Library”.
2. Select Libraries to Include – Highlight libraries related to your sample and press “Add >>”, Press “Ok”, scroll down to the analyze section and press “Search”.
3. Analyze Spectra – Picta also allows spectral mapping of your sample and 3D Mapping. This is accomplished by selecting a peak of interest in the spectral window.

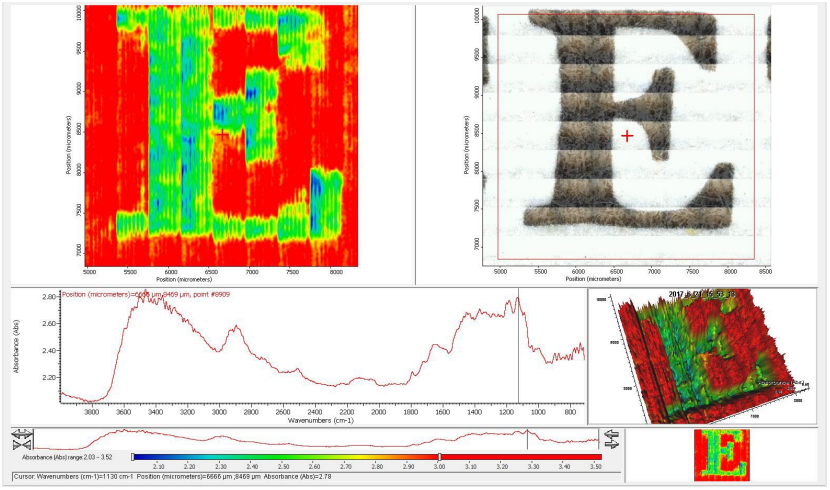


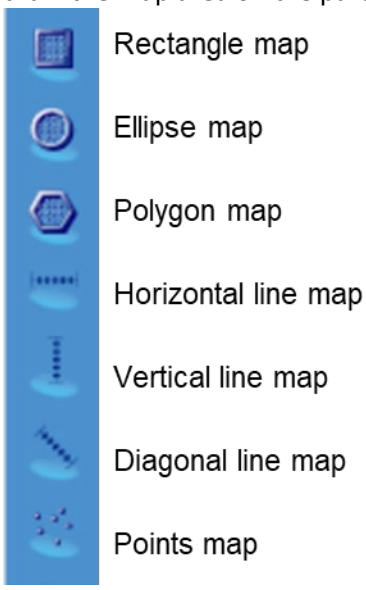
Figure 2. FTIR mapping of printed media.

H. Instrument Shutdown

1. Inspect the Stage – Check to make sure the stage is free of obstructions.
2. Eject the Stage – Press the “Eject” button: to move the stage from the Home position.
3. Remove your Sample – Remove your sample from the holder. Replace the slide with a glass slide, to avoid any dust from getting into the condenser.
4. Return the Stage Home – Press the “Home” button: to return the stage.
5. Turn off the Illumination – Use the illumination sliders to lower the brightness to zero.

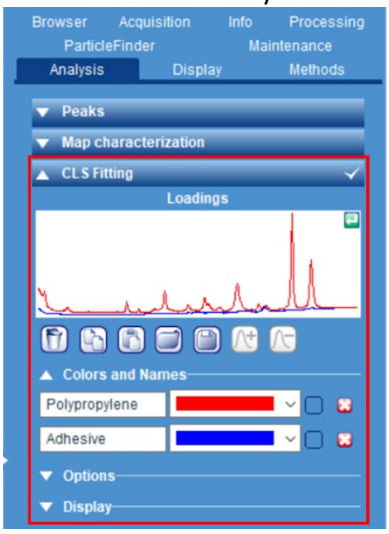
C. Multispectral (Mapping) Data Acquisition and Analysis

1. After visualizing the sample, select the shape of the desired map (e.g., rectangle, ellipse, lines, points), then draw the map area on the particle image.

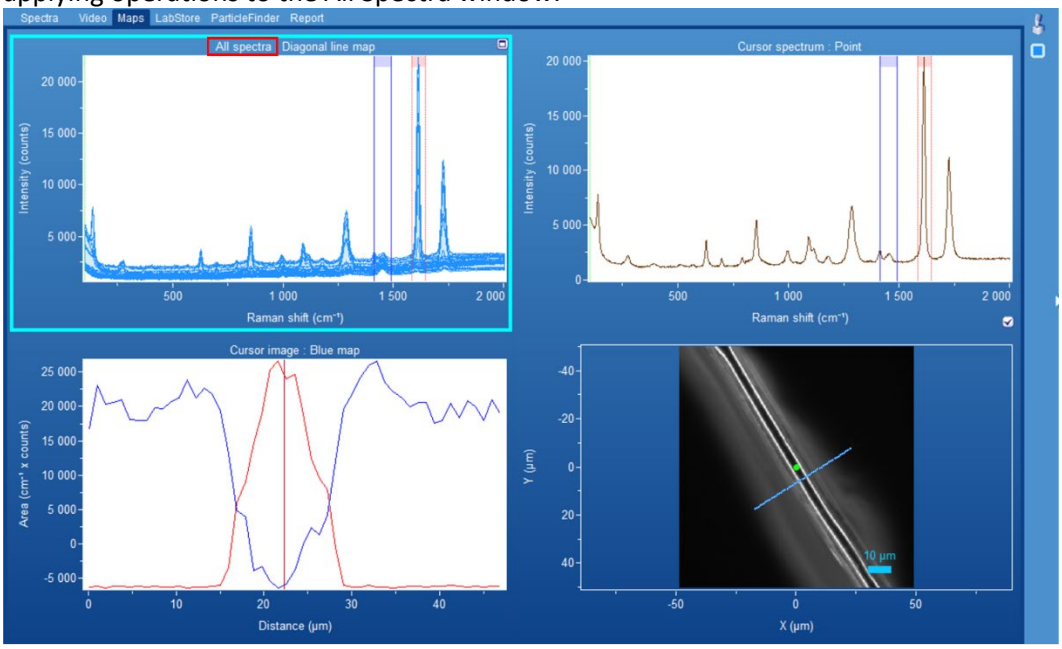


2. Use the same setup parameters defined above for either survey spectra (fast mapping) or spectrum acquisition (high SNR) above.
3. Acquire a Raman map by clicking map acquisition icon. Save the map.

4. Initiate a library search by selecting a spectrum in the map (i.e., a cursor spectrum), and clicking the KnowitAll data link symbol () in the icon bar at the top of the screen.
5. If the sample is completely unknown, explore individual spectra in the map to look for unique spectra.
6. If the sample exhibits visual characteristics of known polymers or products, explore spectra in the map for suspected materials.
7. After identifying unique or suspected spectra, it is possible to classify/identify the rest of spectra in the map based on the similarity to them using classical least square (CLS) fitting (Analysis → CLS).



8. If desired, it is possible to perform baseline correction and smoothing on all spectra in the map at once by applying operations to the All Spectra window.



Note: It is possible to acquire a map over time or along Z axis as well as X- and Y-axes.

Appendix E: Information Management Plan

Trash

Trash data will be submitted through separate data portals depending on the habitat sampled.

Inland Streams

Trash data from inland streams will be submitted through the Stormwater Monitoring Coalition (SMC) Data Portal (https://smc.sccwrp.org/).

For questions and support, please contact Leah Hampton (leahth@sccwrp.org).

Estuaries and Wetlands

Trash data from estuaries and wetlands will be submitted through the Estuary Marine Protected Area (EMPA) Monitoring Program Data Portal (https://empa.sccwrp.org/).

For questions and support, please contact Jan Walker (janw@sccwrp.org) or Leah Hampton (leahth@sccwrp.org).

Epibenthic Marine Debris

Trash data from epibenthic marine trawls will be submitted through the Bight '23 Data Portal (https://bight.sccwrp.org/). Data will be submitted within the Bight 2023 Field section of the data portal. Relevant data tables include Station Occupation (Table 1E), Trawl Event (Table 2E), and Debris (Table 3E).

For questions and support, please contact b23-im@sccwrp.org.

Table 1E. Station Occupation table structure (primary key fields are indicated with bold text).

Field Name	***Type***	***Required***	***Size***	***Description***
StationID	Text	Y	50	A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
OccupationDate	Date/Time	Y		The date the sample was collected expressed as yyyy-mm-dd. All values numeric.
OccupationTime	Text	Y	8	The time of arrival on station expressed in 24hour time (hh:mm:ss).
OccupationTimeZone	Text	Y	10	The time zone of the arrival time. "PST" Pacific Standard Time, "PDT" Pacific Daylight Savings Time, or "NR" for Not Recorded. From lu_TimeZones.
SamplingOrganization	Text	Y	255	The name of the organization doing the sampling. From look-up list lu_Agency.
CollectionType	Text	Y	25	From lu_SampleTypes

Field Name	Type	Required	Size	Description
Vessel	Text	Y	50	The name of the vessel. lu_Vessels
NavType	Text	Y	10	DGPS for differential/GPS for non-differential. From lu_NavTypes. Default = "DGPS"
Salinity	Decimal	*		The field measure of the salinity of the sample water as reported by the instrument expressed in psu or ppt. This is used for estuary samples only.
SalinityUnits	Text		15	Required if Salinity is recorded. See look-up list lu_Units. Default = "ppt".
Weather	Text	Y	35	Field observation of habitat weather from lu_Weather.
WindSpeed	Decimal	Y		Field measurement of habitat wind speed from instrument expressed in knots.
WindSpeedUnits	Text	Y	15	Default = "kts". See look-up list lu_Units.
WindDirection	Text	Y	10	Field observation of wind direction N (North), NE (Northeast), E (East), SE (Southeast), S (South), SW (Southwest), W (West), NW (Northwest), C (calm), NR (Not Recorded). Report in magnetic North. Default = "C". See lu_Directions.
SwellHeight	Decimal	Y		Field Observation of the estimated swell height expressed in feet.
SwellHeightUnits	Text	Y	15	Units the swell height was measured in. Default = "ft". See look-up list lu_Units.
SwellPeriod	Integer	Y		Field observation of the estimated average swell period in seconds. See look-up list lu_Units.
SwellDirection	Text	Y	10	Field Observation of magnetic direction from which the swell travels. N (North), NE (Northeast), E (East), SE (Southeast), S (South), SW (Southwest), W (West), NW (Northwest), C

Field Name	Type	Required	Size	Description
				(calm), NR (Not Recorded). See look-up list lu_Directions.
SeaState	Text	Y	25	Field Observation of sea state. Calm, Rough, Choppy, or Confused from lu_SeaStates.
StationFail	Text	Y	255	From lu_StationFailure. Default value = "None".
Abandoned	Yes/No	Y	3	Was the station abandoned, never to be returned to? Default is "No", but a "Yes" requires a comment.
OccupationDepth	Decimal	Y		The Field Measure of the habitat depth expressed in meters.
OccupationDepthUnits	Text	Y	15	Units the OccupationDepth was measured in. See look-up list lu_Units. Default = "m".
OccupationLatitude	Decimal	Y		Degrees of latitude express in decimal degrees to 5 decimal places (NAD83).
OccupationLongitude	Decimal	Y		Degrees of longitude express in decimal degrees to 5 decimal places (NAD83) expressed as a negative number.
OccupationDatum	Text	Y	50	The datum on which the latitude and longitude are based. The default = NAD83. See look-up list lu_Datum.
Comments	Text		255	Additional comments. Required if Abandoned = "Yes" or for Station Fail Codes that require a comment.

Table 2E. Trawl Assemblage Event table structure (primary key fields are indicated with bold text).

Field Name	*Type*	*Required*	*Size*	*Description*
StationID	Text	Y	50	A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
SampleDate	Date/ Time	Y		The date the sample was collected expressed as yyyy-mm-dd.
SamplingOrganization	Text	Y	255	The name of the organization doing the sampling. From lu_Agency.
Gear	Text	Y	255	Value should be "Trawl" from lu_Equipment.
TrawlNumber	Integer	Y		The sequential number of the trawl at the station. Default = 1.
Datum	Text	Y	50	The datum on which the latitudes and longitudes are based. Default = "NAD83". See look-up list lu_Datum.
OverTime	Text	Y	50	The time the net was deployed expressed as 24-hour time (hh:mm:ss).
OverLatitude	Decimal	Y		Degrees of latitude expressed in decimal degrees to 5 places.
OverLongitude	Decimal	Y		Degrees of longitude expressed in decimal degrees to 5 places and as a negative number.
StartTime	Text	Y	50	The time the net started fishing expressed as 24-hour time (hh:mm:ss).

Field Name	*Type*	*Required*	*Size*	*Description*
StartLatitude	Decimal	Y		Degrees of latitude expressed in decimal degrees to 5 places.
StartLongitude	Decimal	Y		Degrees of longitude expressed in decimal degrees to 5 places andas a negative number.
StartDepth	Decimal	Y		The depth at the start of trawl.
DepthUnits	Text	Y	50	From lu_Units.
WireOut	Integer	Y		The length of wire out expressed in meters.
EndTime	Text	Y	50	The time the net finish fishing expressed as 24-hour time (hh:mm:ss).
EndLatitude	Decimal	Y		Degrees of latitude expressed in decimal degrees to 5 places.
EndLongitude	Decimal	Y		Degrees of longitude expressed in decimal degrees to 5 places and as a negative number.
EndDepth	Decimal	Y		The depth at the end of the trawl in meters.
DeckTime	Text	Y	50	The time the net is recovered and on deck expressed as 24-hour (hh:mm:ss).
DeckLatitude	Decimal	Y		Degrees of latitude expressed in decimal degrees to 5 places.
DeckLongitude	Decimal	Y		Degrees of longitude expressed in decimal degrees to 5 places and as a negative number.

Field Name	Type	Required	Size	Description
TrawlFailure	Text	Y	50	Use to report any trawl fails. Default = “None”. From look-up list lu_TrawlFail.
PTSensor	Yes/No	Y	3	Is there Pressure Temperature Sensor data associated with this trawl? Default = “Yes”.
PTSensorManufacturer	Text		50	Manufacturer of the pressure temperature sensor. Required if a pressure temperature device was used.
PTSensorSerialNumber	Text		50	Tag number listed on PT sensor or generated by user. Required if Pressure temperature device was used.
OnBottomTemp	Decimal	Y		Temperature from the PT sensor.
OnBottomTime	Text	Y	50	Time from PT sensor.
DebrisDetected	Yes/No	Y	3	Was there debris detected in the trawl?
Comments	Text	*	255	Additional comments relative to the trawl. A comment is required for some trawl failure codes.

Table 3E. Trawl Debris table structure (primary key fields are indicated with bold text).

Field Name	Type	Required	Size	Description
StationID	Text	Y	50	A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
SampleDate	Date/Time	Y		The date the sample was collected expressed as yyyy-mm-dd. All values numeric.
TrawlNumber	Integer	Y		The number of the trawl from which the sample was collected.

Field Name	Type	Required	Size	Description
SamplingOrganization	Text	Y	255	The name of the organization doing the sampling. From look-up list lu_Agency.
DebrisType	Text	Y	255	Debris type from lu_DebrisType. Comment required if DebrisType starts with the word "Other".
DebrisCount	Integer	Y*		Number of debris items. Record as -88 if EstimateCategory is used.
EstimateCategory	Text	Y*	15	Only use for Natural Debris whe estimating counts. se for Natural Debris for estimated counts. See data sheet for list of items that can be estimated. Acceptable values include: Moderate=11-100; High = >100. Default = "Not Recorded"
Comments	Text		255	Additional Remarks. Required if DebrisType starts with the word "Other".

Microplastics

Sample Collection

Data pertaining to the collection of samples for microplastics analysis will be submitted through the Bight '23 Data Portal (https://bight.sccwrp.org/). Specifically, data will be submitted through the Bight 2023 Field section of the data portal. For sediment samples, relevant data tables include Station Occupation (Table 1E) and Grab Event (4E).

Table 4E. Grab Event table structure (primary key fields are indicated with bold text).

Field Name	Type	Required	Size	Description
StationID	Text	Y	50	A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
SampleDate	Date/Time	Y		The date the sample was collected expressed as yyyy-mm-dd. All values numeric.
SampleTime	Text	Y	50	The time the sample was collected expressed as 24-hour time (hh:mm:ss).
GrabEventNumber	Integer	Y		Sequential number of each grab.
SamplingOrganization	Text	Y	255	The name of the organization doing the sampling. From look-up list lu_Agency.
Gear	Text	Y	255	From lu_Equipment.

Field Name	Type	Required	Size	Description
Latitude	Decimal	Y		Degrees of latitude expressed in decimal degrees to 5 decimal places (NAD83).
Longitude	Decimal	Y		Degrees of longitude expressed in decimal degrees to 5 decimal places (NAD83) expressed as a negative number.
Datum	Text	Y	50	The datum on which the latitude and longitude are based. The default = NAD83. See look-up list lu_Datum.
StationWaterDepth	Decimal	Y		The field measure of the habitat sample depth expressed in meters.
StationWaterDepthUnits	Text	Y	15	Units the StationWaterDepth was measured in. See look-up list lu_Units.
Penetration	Decimal	Y		Penetration of the grab into the sediment expressed in CM.
PenetrationUnits	Text	Y	15	From lu_Units. The default value is "cm"
Composition	Text	Y	20	Composition of the sediment. The Fraction and units are none. See lu_Composition.
Color	Text	Y	20	Field observation of the Color of the sediment. The default is "Olive Green". The Fraction and units are none. See lu_Color.
Odor	Text	Y	30	Odor of the sediment. The Fraction and units are none. See lu_Odor.
ShellHash	Text	Y	255	Category percentage description: None, Low (1-25%), Medium (26-50%), High (>51%). See lu_ShellHashCategories.
BenthicInfauna	Yes/No	Y	3	Was this grab used for collecting Benthic Infauna?
SedimentChemistry	Yes/No	Y	3	Was this grab used for testing Sediment Chemistry?
GrainSize	Yes/No	Y	3	Was this grab used for testing Grain Size?

Field Name	Type	Required	Size	Description
Toxicity	Yes/No	Y	3	Was this grab used for testing Toxicity?
GrabFail	Text	Y	255	Use to report any grab failures. Default = "None". From lu_GrabFail.
Microplastic	Yes/No	Y	3	Was this grab used for testing Microplastics?
MicroplasticFieldBlank	Yes/No	Y	3	Was a Microplastic Field Blank collected for this grab?
PFAS	Yes/No	Y	3	Was this grab used for testing PFAS?
PFASFieldBlank	Yes/No	Y	3	Was a PFAS Field Blank collected for this grab?
PFASEquipmentBlank	Yes/No	Y	3	Was a PFAS Equipment Blank collected for this grab?
DebrisDetected	Yes/No	Y	3	Was there debris detected in the grab?
Comments	Text	*	255	Additional remarks relative to the grab.

Sample Processing and Analysis

Data pertaining to the processing and analysis of microplastics in sediment and shellfish samples will also be submitted through the Bight '23 Data Portal (https://bight.sccwrp.org/) by September 1st, 2025 (sediment) or April 1st, 2026 (shellfish). Specifically, data will be submitted through the Bight 2023 Microplastics section of the data portal. Relevant tables include the Lab Information (5E), Sample Receiving (6E), Sample Extraction (7E), Instrument Information (8E), Microscopy Settings (9E), FTIR Settings (10E), Raman Settings (11E), and Raw Data Results (12E).

Data templates may be downloaded through the Bight '23 Data Portal (https://bight.sccwrp.org/). For the most up to date descriptions of each data field, please refer to the documentation in the data portal, though descriptions are also provided in the subsequent tables.

For questions and support, please contact b23-im@sccwrp.org.

Table 5E. Lab Information table structure (primary key fields are indicated with bold text).

column name	*datatype*	*required*	*character limit*	*lookuplist table name*	*description*
stationid	**varchar**	**YES**	**255.0**		**A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.**
sampledate	**timestamp**	**YES**			**The date the sample was collected.**
lab	**varchar**	**YES**	**255.0**	**lu_mp_labs**	**Agency analyzing the samples.**
matrix	**varchar**	**YES**	**25.0**	**lu_mp_matrix**	**The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)**
labbatch	**varchar**	**YES**	**50.0**		**Identifier for group of samples processed and analyzed together (1, 2, etc.)**
fieldreplicate	**int2**	**YES**			**Field replicate**
startdate	timestamp	YES			The date the sample was received.
enddate	timestamp	YES			The date that analysis of the last sample was complete.
watertype	varchar	YES	35.0	lu_watertype	Type of water used in the lab for rinsing and mixing (e.g., RO, DI, 1 um filtered).
airfiltration	varchar	YES	3.0	lu_yesno	Is there an air filtration system in the lab?
airfiltrationtype	varchar	NO	255.0		Type of filtration used in the lab (e.g., HEPA filter). Required if AirFiltration is Yes.
sealedenvironment	varchar	YES	15.0	lu_yesno	Is a sealed environment being used to minimize contamination during sample preparation?
sealedenvironmenttype	varchar	NO	255.0		Type of sealed environment (e.g., laminar flow cabinet).
clothingpolicy	varchar	YES	3.0	lu_yesno	Is there a clothing policy in place in the lab?

column name	datatype	required	character limit	lookuplist table name	description
clothingpolicytype	varchar	NO	255.0		Type of clothing policy (e.g., cotton required).
comments	varchar	NO	500.0		Any comments relative to the lab and procedures used.

Table 6E. Sample Receiving table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
stationid	varchar	YES	25.0		A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
sampledate	timestamp	YES			The date the sample was collected
lab	varchar	YES	255.0	lu_mp_labs	Agency analyzing the samples
sampleid	varchar	YES	25.0		The ID assigned to the sample e.g. StationID_MP_SampleType (what was written on the jar)
fieldreplicate	int2	YES			Field replicate
datereceived	timestamp	YES			The date the sample was received
receiver	varchar	YES	100.0		Laboratory personnel who received the samples
matrix	varchar	YES	25.0	lu_mp_matrix	The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)
sampletype	varchar	YES	25.0	lu_mp_sampletypes	Type of sample (Field blank, Lab blank, Result etc)
comments	varchar	NO	255.0		Any comments relative to the samples upon arrival

Table 7E. Sample Extraction table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
sampledate	timestamp	YES			The date the sample was collected
stationid	varchar	YES	25.0		A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
lab	varchar	YES	255.0	lu_mp_labs	Agency analyzing the samples
matrix	varchar	YES	25.0	lu_mp_matrix	The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)
sampletype	varchar	YES	25.0	lu_mp_sampletypes	Type of sample (Field blank, Lab blank, Result etc)
sampleid	varchar	YES	25.0		The ID assigned to the sample e.g. StationID_Matrix_SampleType

column name	datatype	required	character limit	lookuplist table name	description
labbatch	varchar	YES	50.0		Identifier for group of samples processed and analyzed together
fieldreplicate	int2	YES			field replicate
sizefraction	varchar	YES	50.0	lu_sizefraction	Size fraction from lu_SizeFraction (e.g., 125-355 um, 355-500 um, >500 um)
filtertype	varchar	YES	100.0	lu_filtertype	Type of filter used (e.g., PCTE, Cellulose Acetate, Gold Coated, Aluminum Coated, Anodisc)
filterholder	varchar	YES	50.0	lu_filterholder	Glass or Stainless-steel
sievemeshsize_um	int2	YES			Sieve size(s) in um used to extract the microplastics.
sievediameter_in	numeric	YES			The diameter of the sieve in inches
wpodigestions	varchar	YES	50.0		The number of times the size fraction was WPO digested (1:5 ratio of Fe2SO4:H2O2 reached). Please state if one size fraction was put through more rounds of digestion than others
filterporesize_um	int4	YES			The pore size of the filter in um
filterdiameter_mm	int4	YES			The diameter of the filter in millimeters
b1separationtime_hours	numeric	YES			The duration of the first density separation in Beaker 1 (hours)
b2separationtime_hours	numeric	YES			The duration of the second density separation in Beaker 2 (hours)
kohdigestiontime_hours	numeric	YES			Incubation time for KOH digestion (hours)
kohdigestiontemp_c	numeric	YES			Temperature at which digestion took place (Degrees Celsius)
detergentsoaktime_hours	numeric	YES			The duration of the detergent soak (hours)
samplestorage	varchar	YES	3.0	lu_wetdry	Wet (stored in a glass container with RO water) or Dry (stored on a filter paper within a petri dish). If some size fractions are stored differently please state this.

column name	datatype	required	character limit	lookuplist table name	description
timehours	numeric	YES			Time taken for complete sample extraction and size fractioning (hours). Please only include active hands on time (i.e., exclude incubation times, etc.)
comments	varchar	NO	255.0		Additional remarks relative to the sample extraction.

Table 8E. Instrument Information table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
lab	**varchar**	**YES**	**255.0**	lu_mp_labs	**Agency analyzing the samples**
instrumenttype	**varchar**	**YES**	**100.0**	lu_instrumenttype	**Type of instrument used for the analysis. (e.g., stereoscope, FTIR, Raman)**
manufacturer	varchar	YES	255.0		Manufacturer of the instrument
matrix	**varchar**	**YES**	**25.0**	lu_mp_matrix	**The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)**
spectrallibraries	varchar	NO	255.0		Spectral libraries used for spectral matching. In addition to commercial libraries e.g., Bio-Rad, HORIBA, Sigma-Aldrich. Please note all in-house and non-commercial libraries.
librarydetails	varchar	NO	255.0		Provide details of the contents of spectral reference libraries used, in particular any in-house or non-commercial libraries.
softwarecollection	varchar	NO	255.0		Software used for collection of spectra
softwarematching	varchar	NO	255.0		Software used for spectral matching
softwareprocessing	varchar	NO	255.0		Software used for spectral processing (e.g., baseline correction, smoothing)
calibrationfrequency	varchar	NO	255.0		How often is instrument calibrated during sample analysis (e.g., Daily).
comments	varchar	NO	255.0		Any comments relative to the instrument.

Table 9E. Microscopy Settings table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
stationid	varchar	YES	25.0		A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
sampledate	timestamp	YES			The date the sample was collected
lab	varchar	YES	255.0	lu_mp_labs	Agency analyzing the samples
matrix	varchar	YES	25.0	lu_mp_matrix	The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)
sampletype	varchar	YES	25.0	lu_mp_sampletypes	Type of sample (Field blank, Lab blank, Result etc)
fieldreplicate	int2	YES			Field replicate
sampleid	varchar	YES	25.0		The ID assigned to the sample StationID_Matrix_SampleType
labbatch	varchar	YES	50.0		Identifier for group of samples processed and analyzed together
sizefraction	varchar	YES	50.0	lu_sizefraction	Size fraction from lu_SizeFraction (e.g., 125-355 um, 355-500 um, >500 um)
magnification	numeric	YES			Magnification range of the lens used during assessment
pickingprep	varchar	YES	50.0	lu_wetdry	Wet (in a container with RO water) or Dry (on a filter). (see Wet/Dry lookup list)
timehours	numeric	YES			Time taken to sort, pick and characterize (morphology/color) all particles for each size fraction in hours.
comments	varchar	NO	1500.0		Any comments relative to identification via microscopy.

Table 10E. FTIR Settings table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
stationid	varchar	YES	25.0		A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.
sampledate	timestamp	YES			The date the sample was collected
lab	varchar	YES	255.0	lu_mp_labs	Agency analyzing the samples
matrix	varchar	YES	25.0	lu_mp_matrix	The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)
sampletype	varchar	YES	25.0	lu_mp_sampletypes	Type of sample (“Field blank”, “Lab blank”, “Result” etc)
fieldreplicate	int2	YES			Field replicate
sampleid	varchar	YES	25.0		The ID assigned to the sample (StationID_Matrix_SampleType)

column name	*datatype*	*required*	*character limit*	*lookuplist table name*	*description*
labbatch	varchar	YES	50.0		Identifier for group of samples processed and analyzed together
sizefraction	varchar	YES	50.0	lu_sizefraction	Size fraction from lu_SizeFraction (e.g., 125-355 um, 355-500 um, >500 um)
spectracollectionmode	varchar	YES	100.0	lu_spectracollection	The method used to acquire spectra e.g., ATR, reflectance or transmission.
automation	varchar	YES	15.0	lu_yesno	Yes/No for if a method of automated analysis was used for each size fraction. If the choice of automated or manual analysis was decided based on another factor e.g., particle type – please state details in the comments.
accessories	varchar	NO	100.0		Describe any extra accessories, used, besides a filter, for sample presentation and analysis with the instrument.
crystaltype	varchar	YES	100.0	lu_crystaltype	Type of crystal used on the FTIR (e.g., diamond, germanium)
background	varchar	YES	100.0		Measurement of background spectra e.g., before spectrum, after spectrum, every 300 minutes.
spectralrange_cm	varchar	YES	35.0		Wavenumber range of the spectra, reported in cm
spectralresolution	numeric	YES			The maximum number of spectral peaks that the spectrometer can resolve, reported in nm or cm/pixel
spectralresolutionunits	varchar	YES	10.0	lu_spectralresolutionunits	Units for spectral resolution - nm or cm/pixel
numberscans	int2	YES			Number of scans performed.
smoothing	varchar	YES	100.0		Pre-processing step to minimize background noise and interference
baselinecorrection	varchar	YES	100.0		Pre-processing step to flatten baseline and minimize signal interference
datatransformation	varchar	YES	100.0		Spectral data processing technique, often used to normalize signal intensity values.
matchthreshold	numeric	NO			If used, minimum hit quality index (HQI) value for acceptable matches, reported as a percentage.
subsamplingmethod	varchar	NO	255.0		Method used for subsampling, e.g., novel or from literature. Cite

column name	datatype	required	character limit	lookuplist table name	description
					all relevant literature. (Relevant for smallest size fractions where alternative subsampling methods may be used)
timehours	numeric	YES			Time taken to complete FTIR analysis on all subsampled particles, for each size fraction, in hours
comments	varchar	NO	255.0		Any comments related to the analysis of the microplastics via FTIR

Table 11E. Raman Settings table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
stationid	**varchar**	**YES**	**25.0**		**A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.**
sampledate	**timestamp**	**YES**			**The date the sample was collected**
lab	**varchar**	**YES**	**255.0**	lu_mp_labs	**Agency analyzing the samples**
matrix	**varchar**	**YES**	**25.0**	lu_mp_matrix	**The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)**
sampletype	**varchar**	**YES**	**25.0**	lu_mp_sampletypes	**Type of sample (Field blank, Lab blank, Result etc)**
fieldreplicate	**int2**	**YES**			**Field replicate**
sampleid	varchar	YES	25.0		The ID assigned to the sample (StationID_Matrix_SampleType)
labbatch	**varchar**	**YES**	**50.0**		**Identifier for group of samples processed and analyzed together**
sizefraction	**varchar**	**YES**	**50.0**	lu_sizefraction	**e.g., 1-20 um, 20-212 um, 212-500 um, >500 um. (see Size Fraction lookup list)**
spectralrange_cm	varchar	YES	35.0		Wavenumber range of the spectra, reported in cm
spectralresolution	numeric	YES			The maximum number of spectral peaks that the instrument can resolve, reported in nm or cm-1/pixel
spectralresolutionunits	varchar	YES	10.0	lu_spectralresolutionunits	nm or cm-1/pixel
automation	varchar	YES	15.0	lu_yesno	Yes/No for if a method of automated analysis was used

column name	datatype	required	character limit	lookuplist table name	description
					for each size fraction. If the choice of automated or manual analysis was decided based on another factor e.g., particle type - please state details in the comments
field	varchar	YES	50.0		Bright field or dark field
objective	int4	YES			The objective used to analyze particles within each size fraction (e.g., 5x, 50x or 100x)
aperture	varchar	YES	35.0		The aperture of the objective used (e.g., 50-100 um)
spikefilter	varchar	YES	3.0	lu_onoff	Correction of cosmic spikes within the spectra, ON or OFF.
icscorrection	varchar	YES	3.0	lu_onoff	Relative intensity correction of spectra setting, ON or OFF.
smoothing	varchar	YES	100.0		Pre-processing step to minimize background noise and interference
baselinecorrection	varchar	YES	100.0		Pre-processing step to flatten baseline and minimize signal interference (e.g., line, polynomial, manual).
datatransformation	varchar	YES	100.0		Spectral data processing technique, often used to normalize signal intensity values.
laserpower_mw	numeric	YES			The laser power of the instrument should be reported (in mW).
laserwavelength_nm	numeric	YES			e.g., 785nm, 532nm. (must be in nanometers)
lasergrating_nm	int4	YES			e.g., 1200, 600 (must be in nanometers)
matchthreshold	int2	YES			Minimum hit quality index (HQI) value for acceptable matches, reported as a percentage.
matchingprocedure	varchar	YES	100.0		Software matching procedure (e.g., ID Expert or Search It when using Bio Rad KnowItAll software). Where the top match was not chosen, what procedures were used to identify the most accurate match.
subsamplingmethod	varchar	YES	255.0		Method used for subsampling, e.g., novel or from literature. Cite all relevant literature.

column name	datatype	required	character limit	lookuplist table name	description
					(Relevant for smallest size fractions where alternative subsampling methods may be used).
timehours	numeric	YES			Time taken to complete Raman analysis on all subsampled particles, for each size fraction, in hours.
comments	varchar	NO	1000.0		Any comments related to the analysis of the microplastics by the Raman used.

Table 12E. Data Results table structure (primary key fields are indicated with bold text).

column name	datatype	required	character limit	lookuplist table name	description
stationid	**varchar**	**YES**	**25.0**		**A geographic location label as derived from the table of assigned Stations given to each Sampling Organization.**
sampledate	**timestamp**	**YES**			**The date the sample was collected**
lab	**varchar**	**YES**	**255.0**	lu_mp_labs	**Agency analyzing the samples**
matrix	**varchar**	**YES**	**25.0**	lu_mp_matrix	**The sample matrix analyzed (Sediment, Mussel Tissue, Oyster Tissue)**
sampletype	**varchar**	**YES**	**25.0**	lu_mp_sample types	**Type of sample (Field blank, Lab blank, Result etc)**
sampleid	varchar	YES	255.0		The ID assigned to the sample (StationID_Matrix_SampleType)
labbatch	**varchar**	**YES**	**50.0**		**Identifier for group of samples processed and analyzed together**
mass_g_wet	numeric	YES			Measured mass of sediment or tissue (g wet weight)
moisturecontent	numeric	NO			Calculated moisture content (%) of sediment in sample. (Sediment only)
fieldreplicate	**int2**	**YES**			**Field replicate**
sizefraction	**varchar**	**YES**	**25.0**	lu_sizefraction	**Size fraction from lu_SizeFraction (e.g., 125-355 um, 355-500 um, >500 um)**

column name	*datatype*	*required*	*character limit*	*lookuplist table name*	*description*
particleid	**varchar**	**YES**	**75.0**		**The ID assigned to the particle "SampleID_1, SampleID_2, SampleID_3" etc. Particle ID should be represented in the corresponding PhotoID (see below).**
photoid	varchar	NO	75.0		File name of the photo that contains the particle. Required for records where spectroscopy was performed - i.e. the PolymerID is anything other than 'Not measured'
color	varchar	YES	25.0	lu_mp_color	The color of the microplastic particle (see Color Lookup List).
morphology	varchar	YES	25.0	lu_mp_morphology	The shape of the particle (see Morphology Category Lookup List).
length_um	numeric	YES			Length of the particle along its longest axis in microns.
width_um	numeric	YES			Length of the particle along its widest axis perpendicular to length in microns.
stereoscope	varchar	YES	15.0	lu_yesno	Was the particle analyzed using a stereo- or microscope?
ftir	varchar	YES	15.0	lu_yesno	Was the particle analyzed using FTIR spectroscopy?
raman	varchar	YES	15.0	lu_yesno	Was the particle analyzed using Raman spectroscopy?
other_instrument_used	varchar	YES	15.0	lu_yesno	Was the particle analyzed using another instrument not listed?
other_instrumenttype	varchar	NO	255.0		Instrument, that is not already listed, used to analyze particle
ftir_chemicalid	varchar	NO	50.0		Result as shown via chemical ID matching software using FTIR spectroscopy. If unable to identify the chemical ID, then please put "unidentifiable"
raman_chemicalid	varchar	NO	50.0		Result as shown via chemical ID matching software using Raman spectroscopy. If unable to identify the chemical ID, then please put "unidentifiable"

column name	datatype	required	character limit	lookuplist table name	description
other_chemicalid	varchar	NO	15.0		Result as shown via specified instrument. If unable to identify the chemical ID, then please put "unidentifiable"
polymerid	varchar	YES	50.0	lu_polymerid	Broad polymer categories assigned based on FTIR_Chemical ID and/or Raman_Chemical_ID
plastic	varchar	YES	50.0	lu_yesnona	Yes = Chemical and polymer id results show the material is plastic. At present rubber is included in this category. No = Chemical and polymer id results show the material is not plastic. Semi-synthetic fibers e.g., rayon and viscose are currently included in this category. Not Analyzed = The particle was not chemically analyzed therefore we do not have information to say if it was plastic or not.
surrogate	varchar	YES	3.0	lu_yesno	Was the particle a spiked surrogate?
timeimagesmeasurements_hours	numeric	YES			Time taken to manually image and measure all subsampled particles per size fraction, in hours
comments	varchar	NO	1500.0		Additional remarks relative to the sample preparation.

References

(1) Aliani, S.; Griffa, A.; Molcard, A. Floating Debris in the Ligurian Sea, North-Western Mediterranean. *Marine Pollution Bulletin* **2003**, *46* (9), 1142–1149. https://doi.org/10.1016/S0025-326X(03)00192-9.

(2) Amon, D. J.; Kennedy, B. R. C.; Cantwell, K.; Suhre, K.; Glickson, D.; Shank, T. M.; Rotjan, R. D. Deep-Sea Debris in the Central and Western Pacific Ocean. *Frontiers in Marine Science* **2020**, *7*.

(3) Anastasopoulou, A.; Mytilineou, C.; Smith, C. J.; Papadopoulou, K. N. Plastic Debris Ingested by Deep-Water Fish of the Ionian Sea (Eastern Mediterranean). *Deep Sea Research Part I: Oceanographic Research Papers* **2013**, *74*, 11–13. https://doi.org/10.1016/j.dsr.2012.12.008.

(4) Barnes, D. K. A.; Milner, P. Drifting Plastic and Its Consequences for Sessile Organism Dispersal in the Atlantic Ocean. *Marine Biology* **2005**, *146* (4), 815–825. https://doi.org/10.1007/s00227-004-1474-8.

(5) Barnes, D. K. A. Invasions by Marine Life on Plastic Debris. *Nature* **2002**, *416* (6883), 808–809. https://doi.org/10.1038/416808a.

(6) Boerger, C. M.; Lattin, G. L.; Moore, S. L.; Moore, C. J. Plastic Ingestion by Planktivorous Fishes in the North Pacific Central Gyre. *Marine Pollution Bulletin* **2010**, *60* (12), 2275–2278. https://doi.org/10.1016/j.marpolbul.2010.08.007.

(7) Bond, A. L.; Provencher, J. F.; Elliot, R. D.; Ryan, P. C.; Rowe, S.; Jones, I. L.; Robertson, G. J.; Wilhelm, S. I. Ingestion of Plastic Marine Debris by Common and Thick-Billed Murres in the Northwestern Atlantic from 1985 to 2012. *Marine Pollution Bulletin* **2013**, *77* (1), 192–195. https://doi.org/10.1016/j.marpolbul.2013.10.005.

(8) Day, R. H.; Shaw, D. G. Patterns in the Abundance of Pelagic Plastic and Tar in the North Pacific Ocean, 1976–1985. *Marine Pollution Bulletin* **1987**, *18* (6, Supplement B), 311–316. https://doi.org/10.1016/S0025-326X(87)80017-6.

(9) De Frond, H.; O'Brien, A. M.; Rochman, C. M. Representative Subsampling Methods for the Chemical Identification of Microplastic Particles in Environmental Samples. *Chemosphere* **2023**, *310*, 136772. https://doi.org/10.1016/j.chemosphere.2022.136772.

(10) De Frond, H.; Thornton Hampton, L.; Kotar, S.; Gesulga, K.; Matuch, C.; Lao, W.; Weisberg, S. B.; Wong, C. S.; Rochman, C. M. Monitoring Microplastics in Drinking Water: An Interlaboratory Study to Inform Effective Methods for Quantifying and Characterizing Microplastics. *Chemosphere* **2022**, *298*, 134282. https://doi.org/10.1016/j.chemosphere.2022.134282.

(11) Di Beneditto, A. P. M.; Ramos, R. M. A. Marine Debris Ingestion by Coastal Dolphins: What Drives Differences between Sympatric Species? *Marine Pollution Bulletin* **2014**, *83* (1), 298–301. https://doi.org/10.1016/j.marpolbul.2014.03.057.

(12) Farrington, J. W.; Takada, H. Persistent Organic Pollutants (POPs), Polycyclic Aromatic Hydrocarbons (PAHs), and Plastics. *Oceanography* **2014**, *27* (1), 196–213.

(13) Gabrielides, G. P.; Golik, A.; Loizides, L.; Marino, M. G.; Bingel, F.; Torregrossa, M. V. Man-Made Garbage Pollution on the Mediterranean Coastline. *Marine Pollution Bulletin* **1991**, *23*, 437–441. https://doi.org/10.1016/0025-326X(91)90713-3.

(14) Galgani, F.; Souplet, A.; Cadiou, Y. Accumulation of Debris on the Deep Sea Floor off the French Mediterranean Coast. *Mar. Ecol. Prog. Ser.* **1996**, *142*, 225–234. https://doi.org/10.3354/meps142225.

(15) Galgani, F.; Jaunet, S.; Campillo, A.; Guenegen, X.; His, E. Distribution and Abundance of Debris on the Continental Shelf of the North-Western Mediterranean Sea. *Marine Pollution Bulletin* **1995**, *30* (11), 713–717. https://doi.org/10.1016/0025-326X(95)00055-R.

(16) Gall, S. C.; Thompson, R. C. The Impact of Debris on Marine Life. *Marine Pollution Bulletin* **2015**, *92* (1), 170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041.

(17) Goldstein, M. C.; Goodwin, D. S. Gooseneck Barnacles (Lepas Spp.) Ingest Microplastic Debris in the North Pacific Subtropical Gyre. *PeerJ* **2013**, *1*, e184. https://doi.org/10.7717/peerj.184.

(18) Hunt, D.T.E.; Wilson, A.L. The Chemical Analysis of Water: General Principles and Techniques. 2nd Ed. Royal Society of Chemistry. **1986**, London, England 683 pp.

(19) Keller, A. A.; Fruh, E. L.; Johnson, M. M.; Simon, V.; McGourty, C. Distribution and Abundance of Anthropogenic Marine Debris along the Shelf and Slope of the US West Coast. *Marine Pollution Bulletin* **2010**, *60* (5), 692–700. https://doi.org/10.1016/j.marpolbul.2009.12.006.

(20) Kirchner, D. Precision Timekeeping at the Observatory Lustbühel, Graz, Austria. TECHNISCHE UNIV GRAZ (AUSTRIA) DEPT OF COMMUNICATIONS AND WAVE PROPAGATION. **1983**.

(21) Langknecht, T.; Lao, W.; Wong, C. S.; Kotar, S.; El Khatib, D.; Robinson, S.; Burgess, R. M.; Ho, K. T. Comparison of Two Procedures for Microplastics Analysis in Sediments Based on an Interlaboratory Exercise. *Chemosphere* **2023**, *313*, 137479. https://doi.org/10.1016/j.chemosphere.2022.137479.

(22) Lao, W.; Wong, C. S. How to Establish Detection Limits for Environmental Microplastics Analysis. *Chemosphere* **2023**, *327*, 138456. https://doi.org/10.1016/j.chemosphere.2023.138456.

(23) Leggett, C.; Scherer, N.; Curry, M.; Bailey, R. *Assessing the Economic Benefits of Reductions in Marine Debris: A Pilot Study of Beach Recreation in Orange County, California*; Final Report; National Oceanic and Atmospheric Administration, Marine Debris Division, 2014.

(24) Lusher, A. L.; McHugh, M.; Thompson, R. C. Occurrence of Microplastics in the Gastrointestinal Tract of Pelagic and Demersal Fish from the English Channel. *Marine Pollution Bulletin* **2013**, *67* (1), 94–99. https://doi.org/10.1016/j.marpolbul.2012.11.028.

(25) McLaughlin, K.; Mazor, R.; Schiff, K.; Thornton Hampton, L. *Southern California Bight 2018 Regional Monitoring Program: Volume IX. Trash and Marine Debris*; SCCWRP Technical Report 1263; Southern California Coastal Water Research Project: Costa Mesa, CA, 2022.

(26) Moore, S. L.; Gregorio, D.; Carreon, M.; Weisberg, S. B.; Leecaster, M. K. Composition and Distribution of Beach Debris in Orange County, California. *Marine Pollution Bulletin* **2001**, *42* (3), 241–245. https://doi.org/10.1016/S0025-326X(00)00148-X.

(27) Moore, S.; Hale, T.; Weisberg, S. B.; Lorenzo, F.; Kauhanen, P. *California Trash Monitoring Methods and Assessments Playbook*; SFEI Publication #1025; San Francisco Estuary Institute: Richmond, CA, 2020.

(28) Moore, S. L.; Allen, M. J. Distribution of Anthropogenic and Natural Debris on the Mainland Shelf of the Southern California Bight. *Marine Pollution Bulletin* **2000**, *40* (1), 83–88. https://doi.org/10.1016/S0025-326X(99)00175-7.

(29) National Oceanic and Atmospheric Administration, Office for Coastal Management. Coastal Change Analysis Program (C-CAP) Regional Land Cover. Charleston, SC: NOAA Office for Coastal Management. **1995**. www.coast.noaa.gov/htdata/raster1/landcover/bulkdownload/30m_lc/.

(30) Ode, P. R.; Fetscher, A. E.; Busse, L. B. *Standard Operating Procedures (SOP) for the Collection of Field Data for Bioassessments of California Wadeable Streams: Benthic Macroinvertebrates, Algae, and Physical Habitat*; SCCWRP Technical Report 835; Surface Water Ambient Monitoring Program, 2016.

(31) Ribic, C. A.; Dixon, T. A.; Vining, I. *Marine Debris Survey Manual*; NOAA Technical Report NMFS 108; National Oceanic and Atmospheric Administration. file:///C:/Users/leahth/Downloads/noaa_6057_DS1.pdf.

(32) Rios, L. M.; Moore, C.; Jones, P. R. Persistent Organic Pollutants Carried by Synthetic Polymers in the Ocean Environment. *Marine Pollution Bulletin* **2007**, *54* (8), 1230–1237. https://doi.org/10.1016/j.marpolbul.2007.03.022.

(33) Stanley, T.W.; Verner, S.S. The US Environmental Protection Organization's quality assurance program. **1985**, Pp 12-19 In: J.K. Taylor and T.W. Stanley (eds.). Quality Assurance for Environmental Measurements, ASTM STP 867. American Society for Testing and Materials, Philadelphia, PA.

(34) Stefatos, A.; Charalampakis, M.; Papatheodorou, G.; Ferentinos, G. Marine Debris on the Seafloor of the Mediterranean Sea: Examples from Two Enclosed Gulfs in Western Greece. *Marine Pollution Bulletin* **1999**, *38* (5), 389–393. https://doi.org/10.1016/S0025-326X(98)00141-6.

(35) Taylor, J.K. Quality Assurance of Chemical Measurements. **1987**. Lewis Publishers, Inc., Chelsea, MI. 328 pp.

(36) Thornton Hampton, L. M.; De Frond, H.; Gesulga, K.; Kotar, S.; Lao, W.; Matuch, C.; Weisberg, S. B.; Wong, C. S.; Brander, S.; Christansen, S.; Cook, C. R.; Du, F.; Ghosal, S.; Gray, A. B.; Hankett, J.; Helm, P. A.; Ho, K. T.; Kefela, T.; Lattin, G.; Lusher, A.; Mai, L.; McNeish, R. E.; Mina, O.; Minor, E. C.; Primpke, S.; Rickabaugh, K.; Renick, V. C.; Singh, S.; van Bavel, B.; Vollnhals, F.; Rochman, C. M. The Influence of Complex Matrices on Method Performance in Extracting and Monitoring for Microplastics. *Chemosphere* **2023**, 138875. https://doi.org/10.1016/j.chemosphere.2023.138875.

(37) Watters, D. L.; Yoklavich, M. M.; Love, M. S.; Schroeder, D. M. Assessing Marine Debris in Deep Seafloor Habitats off California. *Marine Pollution Bulletin* **2010**, *60* (1), 131–138. https://doi.org/10.1016/j.marpolbul.2009.08.019.

(38) SOP 11: Marsh Plain Vegetation and Epifauna Surveys. https://empa.sccwrp.org/#mp.

(39) *State of Recycling in California*; California Department of Resources Recycling and Recovery (CalRecycle). https://www2.calrecycle.ca.gov/Publications/Details/1706.

(40) *Trash Total Maximum Daily Loads for the Los Angeles River Watershed. California Regional Water Quality Control Board, Los Angeles Region*; CRWQCBLAR, 2007. https://www.waterboards.ca.gov/losangeles/board_decisions/basin_plan_amendments/technical_documents/

2007-012/09_0723/L.%20A.%20River%20Trash%20TMDL_Final%20%20Staff%20Report_August%209,%202007.pdf.

(41) *Santa Monica Bay Nearshore and Offshore Debris TMDL. California Regional Water Quality Control Board, Los Angeles Region*; CRWQCBLAR, 2010. https://www.waterboards.ca.gov/losangeles/board_decisions/basin_plan_amendments/technical_documents/72_New/SMB%20Debris%20Staff%20Report%20073010.pdf.

(42) *Amendment to the Water Quality Control Plan for the Los Angeles Region to Revise the Total Maximum Daily Load for Trash in the Los Angeles River Watershed and the Total Maximum Daily Load for Trash in the Ballona Creek Watershed. California 10 Regional Water Quality Control Board, Los Angeles Region. Resolution Number R15-006*; CRWQCBLAR, 2015. https://www.waterboards.ca.gov/losangeles/board_decisions/basin_plan_amendments/technical_documents/107_new/StaffReportLAandBallonaTrashReconsideration3Apr15.pdf.

(43) *Water Quality Control Policy for Developing California's Clean Water Act Section 303(d) List*; State of California State Water Resources Control Board, 2015. https://www.waterboards.ca.gov/board_decisions/adopted_orders/resolutions/2015/020315_8_amendment_clean_version.pdf.

(44) *Reconsideration of the Santa Monica Bay Nearshore and Offshore Debris TMDL and the Machado Lake Trash TMDL. California Regional Water Quality Control Board, Los Angeles Region*; CRWQCBLAR, 2019. https://www.waterboards.ca.gov/losangeles/board_decisions/basin_plan_amendments/technical_documents/131_New/trashreconsiderationstaffreportSMB121918(rap)posted010819.pdf.

(45) *Statewide Microplastics Strategy*; Ocean Protection Council, 2022. https://www.opc.ca.gov/webmaster/ftp/pdf/agenda_items/20220223/Item_6_Exhibit_A_Statewide_Microplastics_Strategy.pdf.