Sampling and Analysis Plan for Microplastic Monitoring in San Francisco Bay and Adjacent National Marine Sanctuaries

FINAL

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1 Introduction

1.1 Summary of the Issue

Plastic in the ocean, and more specifically microplastic (particles <5 mm), has been gaining global attention as a pervasive and preventable threat to the health of marine ecosystems. Microplastic is ingested by marine organisms (Wright et al. 2013), and may impact their physiological processes (von Moos et al. 2012; Cole et al. 2013, 2015; Rochman et al. 2013, 2014b; Wright et al. 2013; Watts et al. 2015; Lu et al. 2016; Sussarellu et al. 2016). Microplastic may also contain harmful chemicals such as flame retardants, plasticizers, or dyes (Browne et al. 2013; Fries et al. 2013; Rochman et al. 2013, 2014a,b), and may provide a substrate for the adsorption of other harmful chemicals in the ocean, like PCBs and DDT (Teuten et al. 2007), which then may be transferred up the food chain (e.g., Farrell and Nelson 2013; Rochman et al. 2014a; Setala et al. 2014). Many scientific questions remain, however, and there is a need for research on the patterns of distribution and uptake of microplastic by organisms in their ocean ecosystems.

These scientific gaps also exist for San Francisco Bay, where basic questions remain unanswered, such as where, when, and how is microplastic entering the Bay and what circulation patterns deliver them to the ocean. The use of plastic in modern society is ubiquitous; as a result, the pathways by which microplastic reach the Bay, its transport and distribution throughout the Bay, and the levels to which it is taken up into the food web are complex. A preliminary study of nine surface water sites in Central Bay and South Bay showed greater levels of microplastic than in either the Great Lakes or Chesapeake Bay (Sutton et al. 2016). Understanding this stressor is important not only to the health of the Bay, but to the adjacent ocean. In addition, understanding the dynamics of this issue from a scientific perspective is critical to informing and motivating effective policy solutions, interventions, and innovations at the waste treatment, individual behavior, and industrial design level. Current policies that govern wastewater and stormwater treatment processes and current definitions of pollution are inadequate to address this growing and widespread threat. Data are essential to understanding and minimizing the impacts of microplastic on San Francisco Bay and the adjacent ocean.

To develop critical baseline data and inform solutions, the Gordon and Betty Moore Foundation has awarded the San Francisco Estuary Institute and The 5 Gyres Institute a grant for $880,250 to complete a series of studies over 2 years, including water, sediment, and fish monitoring; computer modeling; evaluation of policy options; and communication of findings to the scientific, industry and policy-maker communities as well as the public. The RMP has allocated matching funds of $75,000 and in-kind support for this project. Bay Area stormwater and wastewater agencies are also providing in-kind support through access to sampling areas and expertise.

This document outlines the environmental monitoring that will be conducted to address the data gaps that have been identified for San Francisco Bay and the sanctuaries. The document briefly explains the overall management questions that guide the long-term monitoring for microplastic and provides a context for the goals for this specific project. The document articulates the specific hypotheses and research questions that guided the design of this sampling plan. Additional information on the overarching goals of the microplastic program can be found in the RMP Microplastic Strategy (Sutton and Sedlak 2017).

1.2 Definition of Microplastic

Microplastic is commonly defined as plastic particles smaller than 5 mm (Thompson et al. 2009; Masura et al. 2015). The lower-bound size limit of what is considered microplastic is often operationally defined, with surface water trawl samples typically limited to particles between 5 mm and 355 micron, while other methods can detect smaller particles. Particles smaller than 1 micron are classified as nanoplastic.

Microplastic is a chemically and physically diverse contaminant. The term plastic encompasses materials made up of a broad range of polymers including polyethylene (PE), polypropylene (PP), polystyrene (PS), polyamide (nylon), polyethylene terephthalate (PET or polyester), polyacrylonitrile (PAN or acrylic), polyvinyl chloride (PVC), and styrene butadiene rubber (e.g., vehicle tires) (Hidalgo-Ruiz et al. 2012; Boucher and Friot 2017). Cellulose acetate (i.e., rayon), a non-plastic polymer, is also commonly observed (Andrady 2011). Many of these polymers have significant levels of chemical additives, including flame retardants, plasticizers, and dyes. Plastic polymers and monomers, as well as plastic additives, are the chemical components of microplastic contamination (Fries et al. 2013).

Differences in chemical properties affect the transport of microplastic particles through different environmental matrices. For example, polypropylene and polyethylene are positively buoyant, and float on the surface of the water; polyvinylchloride, polystyrene, polyester and polyamide are high density plastics that are negatively buoyant, likely to sink to the sediment (Anderson et al. 2016).

Microplastic particles come in a broad range of shapes and sizes (Figure 1.1). Through visual observation with the aid of a microscope, particles are commonly classified in five different shape or particle type categories, which in some cases provides insights as to the source of individual particles (Free et al. 2014; McCormick et al. 2014):

- Fragment – hard, jagged particle
- Fiber or line – thin or fibrous, straight plastic
- Pellet – hard, rounded, or spherical particle
- Film – thin plane of flimsy plastic
- Foam – lightweight, sponge-like plastic

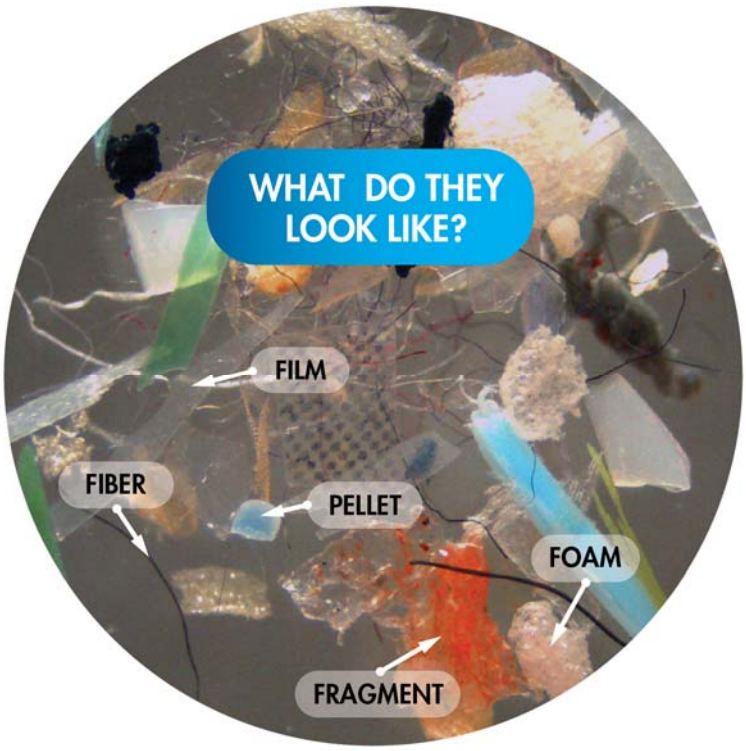
Differences in size and shape can affect the way particles move through the environment, and may modify their potential for toxicity (Wright et al. 2013).

As shown on Table 1.1, this study will evaluate a variety of microplastic size fractions, depending on the matrix under study. Surface water microplastic samples from the Bay and Sanctuaries will be collected using three different methods: Manta trawls, which can capture particles > 355 micron size; a pump, which can capture the 5 mm to 20 micron range; and grab samples, which will be used to determine the nanoparticles < 1 micron. Sediment and fish will be analyzed for particles > 20 micron; a subset of these samples will be analyzed for nanoparticles < 1 micron. Wastewater and stormwater will be analyzed using stacked sieves that cutoff the size fraction at 355 microns (similar to the Manta trawl) and 125 microns. Comparisons of microplastic counts among matrices will only be possible for identical size fractions, determined in part by the sample collection method, as noted above, and in part by subsequent binning of similarly-sized particles when the data is reported.

Table 1.1 Microplastic and Nanoplastic Analyses for Each Matrix

Matrix	Field Collection Method	Microplastic size fraction analysis					Nanoplastic
		> 5 mm	5 mm - 355 µm	355 µm - 125 µm	125 µm - 20 µm	20 µm - 10 µm	
Surface water in Bay + Sanctuary	Manta Trawl	Y	Y				
	Pump with attached screen and filter		Y	Y	Y		Y
Wastewater	Pump with water flow through two sieves			Y			
Stormwater	Pump water flow through two sieves			Y			
Sediment	Grab sample	Y	Y	Y	Y	Y	Y
Fish	Seines		Y	Y	Y	Y	Y

Figure 1.1 Microplastic Particles



1.3 Outline of Major Project Components

This project will support multiple scientific components to develop improved knowledge and characterization of microplastic pollution in San Francisco Bay and National Marine Sanctuaries, including the following:

- Baseline monitoring of microplastic in San Francisco Bay surface water, sediment, and fish.
- Monitoring of microplastic in ocean waters outside of the Golden Gate, providing information on the contribution of Bay microplastic to adjacent National Marine Sanctuaries.
- Characterization of pathways by which microplastic enters the Bay, including wastewater treatment facilities and stormwater.
- Development of an estuarine-marine transport model linking Bay contamination to adjacent Sanctuaries.
- Contributions to standardized sample collection methodology for microplastic in water and common pollution pathways, including wastewater and stormwater discharges.
- Facilitation of evaluation of policy options for San Francisco Bay by leading national and regional experts, with recommendations on source reduction, including potential innovation, design, and household interventions.

- Communication to regional stakeholders and general public through meetings and educational materials.

This Sampling and Analysis Plan describes in detail the following study components: characterization of microplastic in Bay surface water (Section 3), sediment (Section 4) and fish (Section 5); characterization of microplastic outside of the Golden Gate and within adjacent National Marine Sanctuaries (Section 3); characterization of pathways through which microplastic enters the Bay, including wastewater treatment plants (Section 6) and stormwater discharges (Section 7).

The Sampling and Analysis Plan is instrumental for the successful execution of the study, and also supports the goal of advancing methods and tools to standardize how samples are collected and to ensure all major sources of microplastic are captured in system-wide assessments. Method development and standardization can greatly aid in generating and sharing comparable data with regional and global partners in the future.

In addition to advancing methods, the information gathered from the successful completion of the Sampling and Analysis Plan will be used to develop and calibrate models of the transport of microplastic. Microplastic transport modeling will be carried out with particle tracking models, predicting trajectories of virtual microplastic particles as they are transported within the Bay and out into the coastal ocean. The particle tracking will draw on multiple sources for currents using an estuarine hydrodynamic model within the Bay such as SUNTANS or Delft Flexible Mesh, and a combination of a coastal hydrodynamic model such as Regional Ocean Modeling System (ROMS) and observed surface currents outside the Bay.

Lastly, the information gathered in accordance with the Sampling and Analysis Plan will also be used to evaluate policy options for the management of microplastic. Different sources of microplastic will require different management strategies. For example, microfibers are likely derived from synthetic textiles and discharged primarily via treated wastewater. Changes to residential or commercial laundering practices may be an effective means of controlling this source of pollution. A review of possible methods for entraining fibers may be included in the evaluation of policy options, should microfibers be a significant portion of the microplastic identified in the environment.

2 Monitoring Study Design

2.1 Overview of the RMP Microplastic Monitoring and Science Strategy

In 2016, the RMP authorized a special study to develop a strategy for continued study of microplastic in San Francisco Bay. To create this strategy, the RMP convened stakeholders to articulate management questions specific to microplastic pollution, and then conducted a one-day workshop that brought together stakeholders and technical experts to develop an understanding of the state of the science on this emerging contaminant, and determine consensus priorities for future work.

The resulting Microplastic Monitoring and Science Strategy (Sutton and Sedlak 2017) provides:

- an overview of microplastic science relevant to San Francisco Bay,
- the management questions that will guide future work,
- a summary of available sampling and analysis methods, and
- a multi-year plan for studies that would provide answers to the management questions.

The Strategy includes a multi-year workplan that outlines studies in several categories.

- Method development (high priority): Ongoing USEPA method development followed by laboratory intercomparison; ongoing NOAA laboratory intercomparison; additional method development or pilot testing potentially undertaken by RMP, as needed.
- Monitoring biota: Prey fish (high priority); bivalves; sport fish (high priority); benthic organisms.
- Monitoring water and sediment: Ambient and margin sediment (high priority); surface water of Bay and adjacent ocean.
- Characterizing sources, pathways, loadings, and processes: Stormwater and effluent monitoring; transport modeling; refinement of conceptual model.
- Evaluating control options: Evaluating policy options; investigating options for fiber control; characterizing microplastic composition to identify targeted management actions.
- Synthesis: Synthesizing findings, to be presented via symposium.

As noted in the multi-year plan via the “Funder” information, the Moore Foundation grant (along with the RMP contribution) funds the majority of the priority studies recommended for 2017-2019 (Table 2.1).

2.2 Management Questions

Microplastic management questions specific to San Francisco Bay were developed in consultation with RMP stakeholders and external microplastic science advisors and presented via the RMP's Microplastic Strategy document (Sutton and Sedlak 2017). These management questions guide the RMP's microplastic research and monitoring program. These questions are reproduced here to show the overarching goal of the microplastic program and how this project begins to provide information to answer these questions.

The questions are presented in brief below; additional information on the context is presented in the strategy document. However, because the scope of the current undertaking extends beyond the Bay to the surrounding Pacific Ocean, the geographic scope of MQ1 has been broadened for the purposes of this investigation.

MQ1) How much microplastic pollution is there in the Bay and in the surrounding ocean?

This question encompasses two issues: a) selection or development of appropriate methods for characterizing microplastic pollution, and b) presence and abundance of microplastic within the abiotic and biotic Bay and ocean environments. Very few data are available for the Bay; even less information is available for the adjacent ocean.

MQ2) What are the health risks?

This question addresses risks to humans and wildlife from microplastic. Risks to wildlife include physical impacts such as blockages in the digestive tract, as well as impacts associated with chemical exposures from the constituents of plastic or from contaminants sorbed to the plastic. Risks will vary among species, and will also vary with plastic particle shape, size, and composition. Very little information is available regarding toxicity thresholds for microplastic.

MQ3) What are the sources, pathways, loadings, and processes leading to microplastic pollution in the Bay?

This question evaluates the pathways by which microplastic ends up in the Bay. Different sources of plastic produce microplastic particles of characteristic composition and shape or type. Evaluation of potential sources of microplastic may aid in identifying management actions. An evaluation of pathways of microplastic pollution, such as wastewater and stormwater, necessarily involves selection or development of sample collection and analysis methods validated for the matrix, as noted above (MQ1). Loadings of microplastic via these pathways needs to be evaluated alongside other identified pathways, including spills and illegal dumping as well as wind transport, and with the *in situ* process of fragmentation of larger plastic debris to form microplastic. It is also important to understand the fate of microplastic in the Bay, including assessing whether the ocean is a sink or source of microplastic (MQ1).

MQ4) Have the concentrations of microplastic in the Bay increased or decreased?

This question addresses long-term temporal trends, with the specific goal of understanding the forces that lead to any identified trends, including changes in sources (e.g., urban/consumer use of plastic), implementation of management actions relating directly or indirectly to control of plastic or microplastic, and other larger variables such as climate change and drought. Pollution trends may vary with particle size and shape, potentially reflecting different trends relative to sources or pathways.

MQ5) Which management actions may be effective in reducing microplastic pollution?

This question explores alternatives for reducing contamination. Source control is typically found to be the most effective and least expensive pollution prevention option, and may be the primary tool applied to reduce microplastic pollution. The federal ban on plastic microbeads in rinse-off personal care products that will take effect in 2018 is one example of microplastic-specific source control. However, the sources of microplastic to the environment are diverse, and different sources or particle types may be more amenable to source control than others.

These management questions represent overarching goals for the RMP microplastic program. We have designed this sampling plan to address specific questions that are articulated for each matrix. Further discussion of how the results will be used to evaluate specific hypotheses can be found in Section 9.7.

2.3 Data Gaps

A conceptual model of the sources, pathways, processes, and fate of microplastic in and around San Francisco Bay (Figure 2.1) aids in the identification of critical data gaps, many of which will be at least partially filled by this project.

What are the sources of microplastic pollution? What is their relative contribution to the Bay and ocean environment?

Microplastic may be derived from primary or secondary sources (GESAMP 2015). Primary sources are manufactured or enter the environment as particles smaller than 5 mm, and include materials such as pelletized preproduction materials (“nurdles”) that are molded into larger plastic items, or microbeads used as ingredients in consumer products (e.g., exfoliants or toothpastes). In a study of facial scrubs, researchers found that 4,590 to 94,500 microbeads are released in a single use (Napper et al. 2015). Other microplastic that may be introduced into the environment from a primary source includes fibers derived from clothing and textiles made with synthetic material such as polyester or acrylic, or rubber tire particles introduced as a result of abrasion on road surfaces (Boucher and Friot 2017). A single synthetic garment can release 1,900 fibers per wash (Browne et al. 2011).

Secondary sources of microplastic are larger plastic items that disintegrate in the environment through physical fragmentation, photodegradation, chemical weathering, or microbially-mediated biodegradation (Yonkos et al. 2015; GESAMP 2015). Examples of materials that are derived from secondary sources include: plastic fragments from larger plastic items, foam particles from food packaging and cigarette filters, and film from plastic bags and packaging.

Sources anticipated to be relevant to San Francisco Bay are listed in Figure 2.1. Many potential sources have not been characterized at all, including particles derived from abrasive blasting, urban sources such as brake pads and construction materials, or releases via spills or from ships. Of note, spills may include pre-production materials or waste items that escape during the process of waste collection.

The relative importance of these sources to the Bay is unknown. Improved information regarding the relative contributions of sources of microplastic will be particularly useful to regional stakeholders attempting to identify policy solutions to address this contaminant. Rigorous documentation of particles observed in Bay matrices and stormwater and wastewater samples, including relevant information such as particle size, type and polymer composition, will provide evidence connecting a significant portion of the observed plastic to potential sources. However, for a large portion of the collected microplastic, perhaps the majority, there will be insufficient information to link them to original sources.

What are the pathways by which macro- and microplastic enter San Francisco Bay? What is their relative contribution to pollution?

Four major pathways channel plastic pollution to the Bay (Figure 2.1): stormwater discharges; effluent from wastewater treatment plants; wind or airborne particles; and riverine inputs, which can aggregate stormwater, effluent and wind inputs from the greater watershed. Riverine inputs from the Delta, which drains approximately 40 percent of the State, may include inputs from agriculture. In addition, exchange with the Pacific Ocean may introduce some plastic particles to the Bay, though the ocean is likely to be a net sink for this pollution.

To date, limited work has been conducted to evaluate pathways for microplastic entry into the Bay. Based on a review of the literature, it is likely that the two most significant local pathways for microplastic to the Bay are effluent from wastewater treatment facilities discharging to the Bay, and stormwater runoff to the Bay (Anderson et al. 2016). This project will begin to quantify the relative contributions of these two pathways, through monitoring of wastewater and stormwater discharges. In addition, some Bay surface water, sediment, and fish sites have been located near points of discharge of stormwater or wastewater, while others have been placed distant from such influences, as an additional means of exploring the relative impacts of each pathway. Finally, the results of pathways monitoring will be used in a modeling effort designed to predict Bay and coastal California microplastic pollution.

Wastewater treatment facilities are not designed to remove microplastic. Nevertheless, significant removal can occur; a recent mass balance of a European wastewater treatment facility employing

secondary treatment indicated that the plant successfully removed approximately 98% of the microplastic entering the facility (Murphy et al. 2016). Despite high removal efficiency, it was estimated that the facility released 65 million microplastic particles per day via treated effluent to the environment (Murphy et al. 2016). An initial evaluation of Bay Area wastewater treatment plants indicated that microparticles are being discharged in concentrations higher than those observed from similar facilities in the midwestern and northeastern U.S. (Mason et al. 2016). Follow-up study using spectroscopic polymer identification is needed to better understand the potential loadings from this pathway.

At present, there is no information regarding the contribution of microplastic from stormwater to the Bay, although trash monitoring studies have been conducted in local storm drains and demonstrate the ubiquity of larger plastic items within urban litter (e.g., EOA 2014). Storm events likely play a major role in mobilizing macro- and microplastic derived from litter. A southern California study evaluating inputs from the Los Angeles River drainage to the coastal ocean near Long Beach found that concentrations of microplastic increased 7-fold following a storm, from 8 pieces per cubic meter to 56 pieces per cubic meter (Moore et al. 2005). Characterization of this pathway, using methods developed expressly for this purpose, is a critical component of this project.

Of note, wastewater-derived biosolids containing microplastic may be disposed of via land application (Rillig 2012), resulting in contamination of runoff and nearby waterways. Limited application of biosolids occurs on lands near the Bay; this pathway may be particularly important in Central Valley agricultural regions that drain to San Francisco Bay via the Sacramento-San Joaquin Delta.

The Delta, which aggregates wastewater and stormwater inputs from a very large watershed, has not yet been evaluated. Studies of tributaries to the Chesapeake Bay and the Great Lakes suggest that such watersheds can be a significant pathway for microplastic pollution (Yonkos et al. 2015; Baldwin et al. 2016). Surface water monitoring of four tributaries to Chesapeake Bay found microplastic in all but one of the samples collected, with levels ranging from <1 to >560 g/km^2 (Yonkos et al. 2015). The highest concentrations were associated with heavily urbanized areas and with storm events. A study of 29 Great Lakes tributaries found 98% of plastic particles were small enough to be considered microplastic (Baldwin et al. 2016). Fragments, films, foams, and pellets were found at higher levels in tributaries draining urban watersheds, and during conditions leading to runoff, such as rainfall or snowmelt. Interestingly, fibers, the most frequently detected particle type, were not associated with urban areas, wastewater discharges, or runoff (Baldwin et al. 2016).

While this study does not specifically address microplastic contamination from the Delta, samples collected from this region earlier by a key partner, Dr. Chelsea Rochman of the University of Toronto, are likely to be analyzed alongside the samples collected for this project. By leveraging this prior sample collection effort, additional insight may be gained regarding this pathway for pollution. This information will also be helpful for establishing boundary conditions for the model of transport in and out of San Francisco Bay.

What is the level of pollution in San Francisco Bay? How does it compare to other regions of the world? What is the ultimate fate of microplastic in San Francisco Bay?

While the RMP's 2015 special study provided an initial indication of levels of surface water contamination, the number of samples was small and the samples were not subjected to spectroscopic polymer identification essential for quantification of microplastic. In addition, information is entirely lacking on levels of contamination in other Bay matrices such as sediment and biota. Rigorous characterization of spatial and temporal distribution of microplastic within San Francisco Bay matrices is needed to create a baseline from which to assess trends and identify hotspots, to evaluate the potential for bioaccumulation, and to identify possible mitigation measures. It will also be valuable to establish whether Bay levels of contamination are comparable to or greater than levels in other regions. When conducting comparisons, it will be important to identify analytical methods used to extract and determine the microplastic in matrices from other regions, as this may affect the particle count and characterization.

Monitoring results for the Bay must be interpreted in light of geographical and hydrological factors. The North and Central Bays experience frequent tidal flushing and receive freshwater inflows from the Sacramento-San Joaquin River Delta, which likely contain microplastic from upstream watersheds. In contrast, the South Bay receives much lower levels of freshwater inputs and oceanic flushing. Such influences may play a role in the fate and transport of different types of plastic particles. Monitoring data will be used to inform modeling efforts designed to predict microplastic pollution levels within the Bay.

The *in situ* process of fragmentation is also likely to be active and influential in the Bay. After they enter the Bay, plastic particles of all sizes will weather and fragment further; rates are likely to be relatively rapid at the shoreline, but generally several orders of magnitude slower elsewhere, decreasing in the following order: at the sea surface, within the water column, and within sediment (GESAMP 2015). As a persistent substance, plastic is expected to break into smaller particles of microplastic and, eventually, nanoplastic (Andrady 2011). While this project will not directly assess fragmentation, results may indirectly inform our understanding of this process.

Bay monitoring across matrices will also provide information concerning the ultimate fate of microplastic and nanoplastic particles. While some plastic polymers are positively buoyant and tend to float when first released into the environment, many plastics are negatively buoyant, or become less buoyant over time due to growth of biofilm and adsorption of clay minerals (Anderson et al. 2016); these particles are likely to sink to the Bay floor, becoming incorporated into sediment. Some particles will be ingested by biota and incorporated into the food web; these particles may end up in sediment after excretion or when organisms die and sink to the Bay floor. Sediment monitoring is expected to be especially useful for improving our understanding of the fate of these contaminants.

Is the Pacific Ocean a sink or a source of microplastic for the Bay?

Data on microplastic pollution is lacking for coastal California. The Gulf of the Farallones, Cordell Bank, and Monterey Bay National Marine Sanctuaries lie just outside the Golden Gate, providing important commercial and recreational fishing grounds and habitat for protected species. As part of this project, both monitoring and modeling will be used to explore the connection between Bay pollution and the pollution of the adjacent ocean.

Based on the decreasing levels of microplastics from South Bay to Central Bay in the initial RMP study of ambient surface waters (Sutton et al. 2016), it would appear that the Bay is likely a source and the Pacific Ocean is likely a sink for microplastic. However, monitoring within the Sanctuaries is needed to confirm this hypothesis. Factors likely to influence pollution in the Sanctuaries include the complex circulation patterns that move particles within the ocean environment, as well as the diversity of microplastics in terms of shape, buoyancy, and other properties, which may influence their transport over longer distances. Levels of coastal California contamination relative to other regions can inform prioritization of further monitoring and management actions in the Sanctuaries. Monitoring may also identify regional or seasonal variation in contamination with special relevance to protected species residing within the Sanctuaries.

Figure 2.1 Conceptual model of the sources, pathways, processes, and fate of microplastic in and around San Francisco Bay

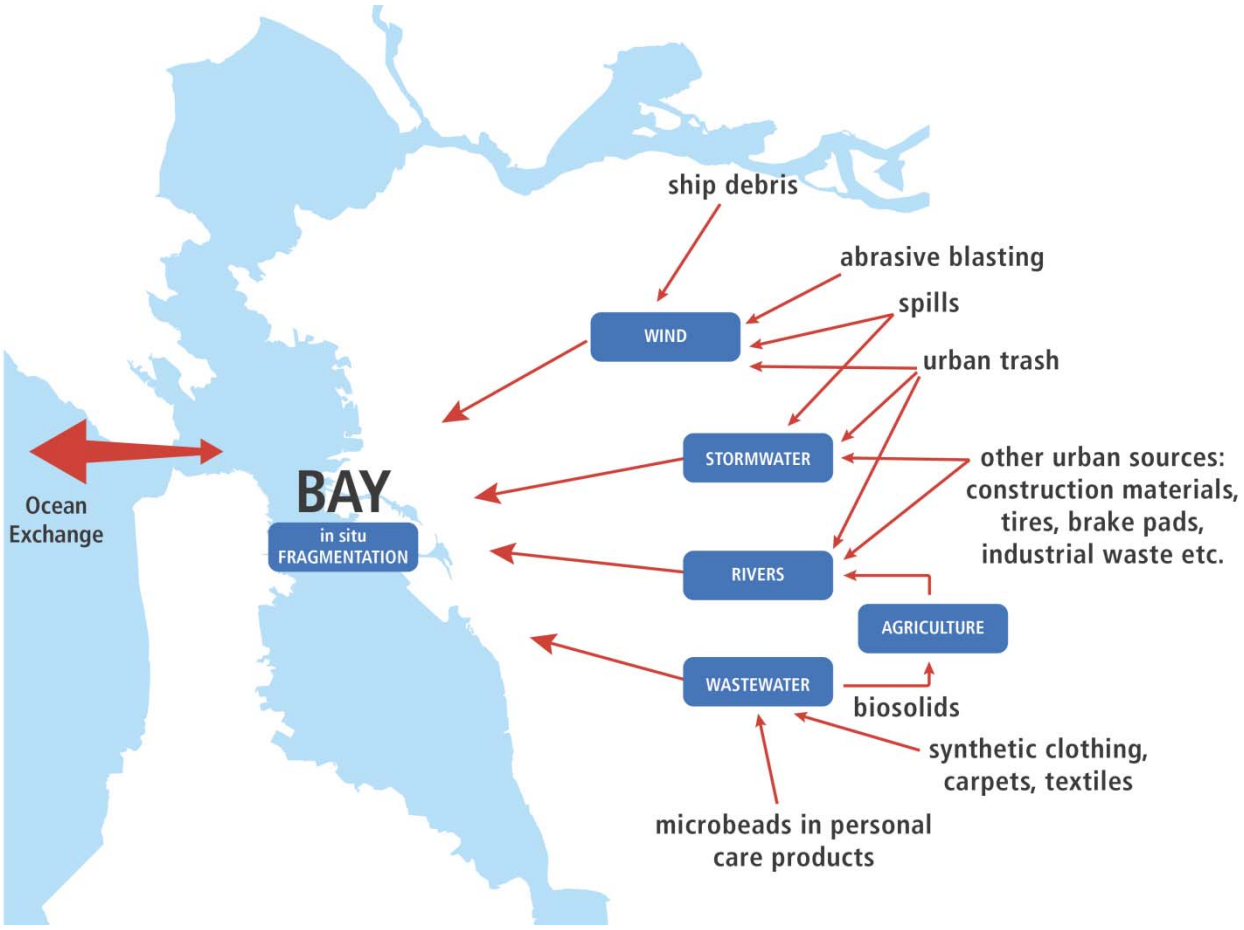


Table 2.1 Microplastic Multi-Year Plan

			Data Gathering and Synthesis			*Long-term Monitoring and Management*		
Task	**Funder**	**Management Questions addressed**	**2016**	**2017**	**2018**	**2019**	**2020**	**Beyond**
Method development [HIGH PRIORITY]	RMP & partner	1 & 3			Additional method development/ pilot testing ($150K)			
	USEPA	1 & 3	New methods for collection, extraction, analysis			Lab inter-comparison ($100K)		
	NOAA	1 & 3	Laboratory intercomparison study					
Monitoring biota [fish HIGH PRIORITY]	RMP	1, 2, 4			Bivalves ($50K)	Sport fish ($190K)	Bivalves ($50K)	Benthic organisms ($50K)
	Moore Fdn with RMP	1, 2, 4		Prey fish ($130K)				
Monitoring water and sediment [surface sediment HIGH PRIORITY]	Moore Fdn with RMP	1, 3, 4		Archived ambient & margin sediment ($100K)				
	Moore Fdn with RMP	1, 3, 4		Surface water ($100K)				
	Moore Fdn with RMP	1, 3, 4		Surface water of adjacent ocean ($120K)				
	RMP	1, 3, 4						Sediment cores ($50K)
Characterizing sources, pathways, loadings, processes	RMP	1 & 3				Refine conceptual model ($50K)		
	Moore Fdn with RMP	1 & 3			Stormwater & effluent ($90K)			
	Moore Fdn with RMP	1 & 3			Model transport in Bay and ocean ($80K)			
Evaluating control options	Moore Fdn with RMP	5				Evaluating policy options ($40K)		
	RMP & partner	5					Options for fiber control ($40K)	
	external	5						Characterize microplastic composition to identify management actions ($60K)
Synthesis	Moore Fdn with RMP	1 & 3				Synthesize findings, hold symposium ($220K)		

3 Surface Water: San Francisco Bay and Adjacent Sanctuaries

3.1 Objective

The objective for this element is to quantify the levels and composition of microplastic and nanoplastic pollution in surface water samples in San Francisco Bay and San Francisco Bay's adjacent National Marine Sanctuaries (e.g., Greater Farallones, Cordell Bank, Monterey Bay) during dry and wet seasons using a range of collection and analysis methods. At present, there are limited microplastic and nanoplastic data within San Francisco Bay and the adjacent National Marine Sanctuaries (Sutton et al. 2016; Doyle et al. 2011). The RMP's Microplastic Monitoring and Science Strategy (Sutton and Sedlak 2017) identified the need for additional data on microplastic levels in surface waters in San Francisco Bay. In addition, the lack of data to determine whether the Bay is a sink or a source of microplastic particles to the nearby Pacific Ocean was noted. Therefore, this study aims to collect baseline data on microplastic and nanoplastic floating on surface waters in and adjacent to San Francisco Bay.

While microplastic has been found to be a ubiquitous contaminant of aquatic environments, limited monitoring has been conducted in San Francisco Bay. In a 2015 RMP special study, nine Bay surface water samples were collected from the central and southern portions of the Bay during the wet season and examined for microplastic. Detailed methods and findings are provided in Sutton et al. (2016). With an average particle abundance of 700,000 particles/km^2, Bay surface water appeared to have higher microplastic levels than other urban water bodies sampled in North America, such as the Great Lakes and Chesapeake Bay (Eriksen et al. 2013; Yonkos et al. 2014; Sutton et al. 2016). Higher San Francisco Bay microplastic levels may be partially explained by high population density as well as the limited interchange of Bay waters with the Pacific Ocean.

As a dense urban metropolis surrounding a semi-enclosed water body, San Francisco Bay is an ideal laboratory for investigations of microplastic. However, large data gaps remain regarding microplastic pollution, motivating focused attention from the RMP and others. In addition, limitations in the method used in the original 2015 study, specifically the lack of definitive polymer identification via spectroscopic means, limit the conclusions that can be supported by the results. Further characterization of spatial and temporal distribution of microplastic within San Francisco Bay is needed to assess trends, to identify hot spots, to evaluate the potential for bioaccumulation, to identify possible mitigation measures, and to evaluate the effectiveness of measures such as the microbead ban.

The newly expanded Gulf of the Farallones, Cordell Bank and Monterey Bay National Marine Sanctuaries lie just outside the Golden Gate, and are home to several protected and important species including birds, mammals, and sharks; as well as important commercial and recreational fishing grounds. However, there have been limited studies of microplastic in the sanctuaries (Doyle et al. 2011).

Hydrological factors may play a role in levels of microplastic contamination in different parts of the Bay and in the adjacent sanctuaries. The North and Central Bays experience frequent tidal flushing and

receive freshwater inflows from the Sacramento-San Joaquin River Delta, which likely contain microplastic from upstream watersheds. In contrast, the South Bay receives much lower levels of freshwater inputs and oceanic flushing. The entire San Francisco Bay drains into the Pacific Ocean under the Golden Gate, distributing microplastic into the adjacent National Marine Sanctuaries at unknown levels.

Microplastic and nanoplastic likely occur throughout the entire water column in San Francisco Bay (Anderson et al. 2016); however, this study will focus on sampling surface waters (as described in this section) and sediment (as described in Section 4). Very little is known about the profile of microplastic in the water column. One study evaluating this issue indicated that there was an exponential decline in microplastic concentrations with depth; it was also noted that Manta trawls likely underestimate the amount of microplastic present (Kooi et al. 2016).

Surface water microplastic and nanoplastic samples will be collected using three methods at sampling sites identified throughout San Francisco Bay and within the sanctuaries. Microplastic samples will be collected using the traditional method with a Manta trawl (Eriksen et al. 2013; Free et al. 2014; Masura et al. 2015), a winged, rectangular metal box open on the ends that funnels surface water debris into a net with a fine mesh (typically ~ 0.3 mm), allowing for the characterization of microplastic greater than 355 microns. Thirty-minute surface trawls will be undertaken in the Bay and sanctuaries. Recent research suggests that Manta trawls may underestimate the amount of microplastic present, in part because the smaller microplastic fraction is not captured by the trawl (Barrows et al. 2016). To assess smaller fractions of microplastic, a pump system will be used to collect particles 20 microns and larger at approximately half of the sites included in the project. Ten liters of surface water will be pumped through a 20-micron filter for analysis.

To quantify nanoplastic particles, grab samples of one liter of surface water will be collected at each site and plastic particles including those less than one micron will be analyzed in the lab. If insufficient concentrations of nanoplastic are present in the one-liter grab samples for analysis, alternative methods to filter surface water will be explored analogous to those for isolating microplastic.

This work will be conducted in the summer of 2017 and winter 2017/2018.

3.2 Relevant Management Questions

This element, monitoring microplastic and nanoplastic in the surface waters of San Francisco Bay and adjacent Marine Sanctuaries, provides information useful for addressing four of the management questions listed previously:

- MQ1. How much microplastic pollution is there in the Bay and the surrounding ocean?
- MQ3. What are the sources, pathways, loadings, and processes leading to microplastic pollution in the Bay?

- MQ4. Have concentrations of microplastic in the Bay increased or decreased?
- MQ5. Which management actions may be effective in reducing microplastic pollution?

Characterization of baseline data on microplastic and nanoplastic in San Francisco Bay and the ocean sanctuaries surface waters using appropriate methods is essential to help answer MQ1. This study will build from the initial pilot project carried out in 2015 that found microplastic in San Francisco Bay surface water (Sutton et al. 2016). The more robust characterization carried out as part of this project will provide a baseline for evaluating temporal trends (MQ4), should monitoring be replicated in the future.

To better understand the fate and transport of microplastic within the Bay and the exchange with the adjacent Pacific Ocean, a model of microplastic transport will be developed as part of this project (Section 3.3) to help answer MQ3; data from this project will help to calibrate and validate the model. The model will help to refine the conceptual model for the transport of microplastic (Figure 2.1). In addition, baseline data on microplastic and nanoplastic within the adjacent National Marine Sanctuaries will better understand how microplastic moves through the ecosystem and what types of microplastic are transported into adjacent waters.

Each microplastic and nanoplastic sample will be analyzed for particle size, type (e.g., fragment, fiber, etc.) and polymer composition (e.g., polyethylene, polypropylene, etc.). This information will be useful for characterizing possible sources, determining the magnitude of the problem, and informing possible management solutions (MQ 5).

These management questions present overarching goals for the RMP microplastic program. We have designed the sampling plan for water for this project based on specific questions that are articulated in the following section. Further discuss of how the results will be used to evaluate these hypotheses is discussed in Section 9.7.

3.3 Site Selection

The selection of sites to sample the surface waters within San Francisco Bay and the adjacent National Marine Sanctuaries was driven by the following questions.

- Are levels of microplastic and nanoplastic higher in San Francisco Bay than in the adjacent National Marine Sanctuaries?
- Is the composition of microplastic and nanoplastic different within San Francisco Bay than in adjacent sanctuaries (e.g., morphology and/or chemical composition)?
- Do we see microplastic and nanoplastic particle patterns or signatures (e.g., shape, particle type, and/or chemical composition) of specific pollution pathways (e.g., effluent and stormwater) in samples collected near discharges? Do we see signatures of these sources in the microplastic collected from the ambient Bay?

Tables 3.1 and 3.2 include a summary of the sampling design for microplastic and nanoplastic surface water samples in the San Francisco Bay (Table 3.1) and National Marine Sanctuaries (Table 3.2), including a list of monitoring sites and the number and type of surface water samples to be collected at each. All monitoring sites will be sampled during dry and wet weather events, resulting in two samples from each site. Maps of the water sampling sites are shown in Section 8. Further details on the exact sampling sites, target site coordinates, and rationale for site selection are provided in Appendix B-1.

Spatial Representation

As mentioned above, there are many factors that can influence the levels of microplastic and nanoplastic contamination within San Francisco Bay and within the adjacent National Marine Sanctuaries.

The hydrological differences throughout the project area, combined with outfalls and tributaries, can influence microplastic contamination levels to varying degrees. To address the multiple factors, samples will be collected in four segments of San Francisco Bay, including Lower South Bay, South Bay, Central Bay, and North Bay (including Suisun Bay and San Pablo Bay), and within the three adjacent National Marine Sanctuaries (Greater Farallones, Cordell Bank, Monterey Bay). Figure 8.1 shows the geographic boundaries of these areas. The numbers of samples are almost evenly distributed by segment within the San Francisco Bay; however, additional samples will be collected in the Central Bay because the geographic area is larger than South Bay and North Bay. Within each segment, 3-6 samples are distributed and placed near wastewater outfalls, tributaries, and in ambient locations closer to the spine of the Bay and more distant from these pathways. We are interested in evaluating whether there is a difference between samples collected near outfalls and stormwater versus samples collected in more ambient locations. It is likely we will have too few samples to provide rigorous statistical analysis regarding this particular question. Nonetheless, it may be possible to make inferences if the source compositions (i.e. storm water and wastewater) are distinctly different. A discussion of data analyses and statistical methods is presented in Section 9.7.

Four samples will be collected from each of the National Marine Sanctuaries. Several of the samples within the Monterey Bay and Greater Farallones Sanctuaries are located around in close proximity to the Golden Gate to characterize flow from the San Francisco Bay. Monterey Bay Sanctuary extends south to San Luis Obispo County, but all sampling will be conducted north of Pedro Point in Pacifica, CA. We will evaluate the difference in microplastic concentrations and composition between the sanctuaries and the Bay using statistical methods that are described in Section 9.7.

Seasonal Characterization

The Bay and sanctuary waters will be monitored in both the dry and wet seasons to assess seasonal variability. California has a Mediterranean climate in which precipitation occurs largely during the winter months; rain rarely occurs in the summer months. The dry season is defined for this project as June through September. In the unlikely event that rain occurs during the dry season, monitoring would occur

at least one week after a precipitation event (greater than 0.5 inches in 24 hours). The wet season is defined for this project as November through April. Monitoring for a wet weather event will be determined through best professional judgment in consultation with the modeling team for this project and the vessel captain. To determine whether there has been a storm system of sufficient magnitude to affect the Bay and adjacent sanctuaries, the team will consider: Delta outflow; the magnitude and number of storm events leading up to proposed wet weather sampling; and the salinity of the Bay. It is anticipated that Bay sampling would likely be undertaken within 3 days after a major storm event.

Given the areal extent of the National Marine Sanctuaries, sampling should occur at least 5 days after a major storm system to allow transport of microplastic out of the Golden Gate and mixing within the sanctuaries. Ideally the sampling will be taken within 10 days after a storm. Mobilizing for wet weather sampling will be a function of availability of vessels and may require some deviation from the proposed criteria. If this is necessary, the field staff will discuss possible options with the microplastic external advisers and the modeling team.

Statistical methods to evaluate the difference in microplastic concentrations and composition in water from wet and dry season is discussed in Section 9.7.

Modeling Needs

To better understand the fate and transport of microplastic within the Bay and the exchange with the adjacent Pacific Ocean, a key element of the overall project is development of a model of microplastic transport. Microplastic transport modeling will be carried out using particle tracking models to predict trajectories of virtual microplastic particles. As noted previously, the particle tracking will draw on multiple sources for currents using an estuarine hydrodynamic model within the Bay, such as SUNTANS or Delft Flexible Mesh, and a combination of a coastal hydrodynamic model such as Regional Ocean Modeling System (ROMS) and observed surface currents outside the Bay. The modeling team has participated in the selection of water sampling sites described above, to ensure that the monitoring data generated are optimized for the modeling effort.

3.4 Field Sample Collection Methods

This project will employ conventional field methods for the larger size fraction of microplastic (e.g., Manta trawls for the capture of microplastics > 355 microns); and will develop new methods for collection of the smaller fractions of microplastics and nanoplastics. Limited field methods exist to collect and analyze microplastic below 355 microns; however, this study will build off of the small number of available studies to help standardize sample collection.

Microplastic Samples (355 micron and above)

The Manta trawl, a modified Neuston net with a rectangular opening of 16 cm high by 61 cm wide, aluminum frame, and a 3 m long, 5 mm net with 30 x 10 square cm collecting bag, will be used to collect microplastic samples (355 micron and larger) from surface waters (Eriksen et al. 2013; Free et al. 2014;

Masura et al. 2015). The trawl will be towed behind a vessel for 30 minutes at each site, with tow speeds below 3 knots, while vessel maintains a consistent heading. A flow meter is attached to the trawl to record how much water passes through trawl, allowing for calculation of standardized values per square kilometer. Plastic pollution samples captured in the cod end of the net will be placed in a clean sample jar.

Microplastic Samples (20 micron to 5 mm)

A pump system is being designed and built for the project that consists of a 20-micron filter to capture particles from approximately 10 liters of surface water (Lusher et al. 2015; Talvitie et al. 2017; USEPA 2013). The pump system has a 5 mm pre-filter on the end of the ¾ inch tube that is placed in the water (top 12 inches of water column). The pre-filter prevents the 20-micron filter from clogging. Water is pumped through the 20-micron filter using an attached hand-pump. A flowmeter on the system tracks the amount of water passing through the filter, allowing for calculation of standardized values per liter of water. For transport, each filter will be placed in a clean glass petri dish with a lid and sealed (taped around the entire lid).

Nanoparticle Samples (< 1 micron)

A 1-liter glass sample jar will be filled with surface water at each site to be analyzed for nanoplastic (Barrows et al., 2016). Prior to taking each sample, the clean sample jar will be rinsed with surface waters three times before the bottle is filled and capped. This procedure may be modified if the analytical laboratory determines that a larger sample is required to identify nanoplastics.

Data collection will take place onboard a sailing vessel by SFEI and 5 Gyres staff. Approximately 8-12 samples will be collected per day (e.g., 3 to 4 manta trawls, 3 to 4 nanoplastic grab samples, and 2-3 pump samples). It is possible due to inclement weather or equipment malfunctions that fewer samples will be collected per day; an effort will be made to increase the number of stations on a separate day so that we can still meet our scheduled cruise dates. Tables 3.1 and 3.2 present the number of each type of sample that will be collected. Appendix B-1 includes the field collection information and data sheets.

Quality Assurance / Quality Control Samples

As shown on Table 3.1 and 3.2, field blank samples will be collected to assess the potential for the introduction of microplastic during sample collection and transport; field duplicates will be taken to assess variation. Field blanks consist of DI water filtered through the Manta trawl or the 20-micron pump that is grouped with the field samples and processed using the same field methods. Two Manta trawl blanks will be collected in the Bay and in the sanctuaries per season (4 total). One field duplicate will be collected in the Bay and in the Sanctuary by conducting two consecutive trawls at the same location. Field blanks, along with field duplicate samples, will be collected for the pump and nanoplastic analyses as well. Further details related to the collection of field blanks are presented in Appendix B-1, Table B-1.3.

3.5 Laboratory Analyses

Microplastic Analyses (20 micron and larger)

Manta trawl and pump system samples will be processed and analyzed for microplastic by the University of Toronto Rochman laboratory. Organic content in the samples will be digested using a 4N KOH solution at room temperature for 14 days. These are methods that have been tested against other extraction options and are cited as the most effective at removing organic material without compromising the integrity of the microplastic (Dehaut et al., 2016; Lusher et al., 2016). After digestion, samples will be filtered through a 10 um polycarbonate filter and sealed to prevent cross-contamination. Filters will be analyzed via Raman spectroscopy using Particle Finding software that scans each suspect particle across the filter. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, morphology, and chemical composition will be reported. Photographs of the particles will also be included in the data package. Further discussion of methods is presented in Appendix C.

Nanoparticle Analysis (< 1 micron)

Surface water samples will be analyzed for nanoparticles by the Duhaim/Banaszak Holl Laboratory at the University of Michigan. This research group will develop and implement a method to detect nanoplastic from one-liter grab samples and/or larger volumes of filtered water. The analytical approach will feature a combined application of infrared (IR) spectroscopy and atomic force microscopy (AFM). The AFM-IR technology will simultaneously provide information about particle morphology and chemical content for particles ranging from tens of microns to tens of nanometers. Information on particle size, morphology, and chemical composition will be reported. In addition, images of particles will be submitted with the data package. If fields of particles are observed, statistical data will be provided; an example is provided in Merzel et al. 2016. This technology, photothermal infrared spectroscopy (PTIR) or AFM-IR spectroscopy, can be used to directly collect IR spectra on a particle-by-particle basis, and even map variations in content within a particle. This capability is important to characterize bi-component particles and fibers and polymer blends. The spatial resolution (~10 – 50 nm) is substantially better than IR microscopy (~2000-5000 nm) and is a nice complement to Raman spectroscopy because it is not compromised by the presence of dyes or strongly absorbing fillers.

AFM-IR has been successfully applied to spatially map the chemical content of diverse materials, including industrial application for the identification of polymeric and biological materials. The University of Michigan laboratory also has an FT-IR microscope and a Raman microscope if complementary methods are required. Further discussion of methods is presented in Appendix C.

Quality Assurance / Quality Control

The field of environmental microplastic analysis is in its infancy and it is not clear that we can translate the same techniques that we use for analytical chemistry for typical environmental contaminants to microplastic. A goal of this project is to develop more standardized methods and techniques and to assure that we are not introducing contamination during sampling and laboratory analyses. Although

NOAA has developed a method for analyzing microplastic (Masura et al. 2015), their method has several shortfalls that have been identified (see for example Dyachenko et al. 2016) and methods for analyses of microplastic continue to improve (see for example Dehaut et al. 2016; Corcoran et al. 2015). The laboratory processing methods and laboratory analyses that are being employed as part of this project have been shown to perform well (Dehaut et al. 2016; Lusher et al., 2016). For nanoparticles, this project is very much a proof of concept that will focus on developing methods for surface water, sediment and fish.

In general, laboratory QA/QC will involve best practices (i.e., wearing cotton clothing, filtering in a clean space and not allowing samples to be exposed to the air when possible, using clean glassware and ultrapure water) for reducing procedural contamination, and will use laboratory and field blanks to account for any procedural contamination. In the laboratory, one laboratory blank sample will be run each day that the lab performs extractions. For batches of more than 10, there will be one lab blank per 10 samples. Lab blank samples will be filtered DI water that has gone through the same procedure for extraction and analyses as the field samples, i.e., digested in KOH, filtered and analyzed via Raman spectroscopy. Microplastic in the field and lab blank samples will be characterized and microplastic of the same type and morphology will be subtracted (blank corrected) from the field samples to account for procedural contamination. The results of the blanks will be reported for all analyses.

Table 3.1. Total number of microplastic and nanoplastic samples to be collected during dry and wet weather sampling at San Francisco Bay monitoring sites											
Location	**Monitoring Site**	**Subregion**	**# Manta Trawl Samples**	**# Manta Trawl Blanks**	**#Manta Trawl Field Duplicates**	**# Nano Samples**	**# Nano Blanks**	**#Nano Field Duplicates**	**# 20 Micron Pump Samples**	**# 20 Micron Pump Blanks**	**# 20 Micron Pump Duplicates**
Lower South Bay	MMP - LSB – GR	Guadalupe River	2		2	2		2	2		2
	MMP - LSB – PA	Near Palo Alto WWTP	2			2					
	MMP - LSB – MAIN	Main stem of LSB	2			2			2		
South Bay	MMP - SB – SM	San Mateo Creek	2			2			2		
	MMP - SB - MAIN – SE	South Bay main - Southeast	2		2	2					
	MMP - SB - MAIN – NE	South Bay main - Northeast	2			2					
	MMP - SB - MAIN – SW	South Bay main - Southwest	2	2		2	2		2	2	
Central Bay	MMP - CB – BAYBRIDGE	South of Bay Bridge	2			2					
	MMP - CB – SL	San Leandro Creek / Oakland Airport	2			2			2		
	MMP - CB – EMERY	Emeryville	2			2					
	MMP - CB – RICH	South of Richmond/ San Rafael bridge	2			2					
	MMP - CB – OUTFALL	Near EBDA outfall	2			2			2		
	MMP - CB – ANGEL	Central Bay main, Southeast of Angel Island	2			2					
North Bay	MMP - NB – PR	Petaluma River	2			2			2		
	MMP - NB – SUISUN	Suisun Bay main	2			2					
	MMP - NB – SANPABLO	San Pablo Bay main	2	2		2			2		
TOTAL			**40**			**36**			**20**		
TOTAL SAMPLES: 96											

Table 3.2. Total number of microplastic and nanoplastic samples collected during dry and wet weather sampling at National Marine Sanctuary monitoring sites

Location	Monitoring Site	Subregion	# Manta Trawl Samples	# Manta Trawl Blanks	# Manta Trawl Field Duplicates	# Nano Samples	# Nano Blanks	# Nano Field Duplicates	# 20 Micron Pump Samples	# 20 Micron Pump Blanks	# 20 Micron Pump Field Duplicates
Cordell Banks	MMP - CORD - S	Southern Edge	2			2			2		
	MMP - CORD - N	Northern Edge	2			2			2		
	MMP - CORD - E	Eastern Edge	2			2					
	MMP - CORD - W	Western Edge	2			2					
Greater Farallons	MMP - FAR - C	Farallon Islands	2	2	2	2	2	2	2	2	2
	MMP - FAR - GG	At discharge of GG, SF Plume	2			2	2		2		
	MMP - FAR - S	Off of Point Reyes	2			2					
	MMP - FAR - W	Western Edge	2			2					
Monterey Bay	MMP - MONT - GG	At discharge of GG, SF Plume	2			2			2		
	MMP - MONT - OB	At discharge of GG, SF Plume	2			2			2		
	MMP - MONT - S	Off coast of Ano Nuevo	2			2					
	MMP - MONT - W	Western Edge	2			2					
TOTAL			**28**			**28**			**16**		
TOTAL SAMPLES: 72											

4 San Francisco Bay Sediment

4.1 Objective

The objective for this element is to quantify the concentration and composition of microplastic and nanoplastic in sediment. At present, there are no microplastic or nanoplastic sediment data for San Francisco Bay; this lack of data is identified as a significant gap in the Microplastic Monitoring and Science Strategy (Sutton and Sedlak 2017) and was given a high priority at the June 2016 RMP Microplastic workshop.

3. Characterizing these contaminants in Bay sediments is important for several reasons. First, sediment may be a source of microplastic and nanoplastic to the food web. Benthic dwelling organisms such as crustaceans and bivalves may ingest sediment containing microplastic (Wright et al 2013). Predators such as fish and birds may consume benthic dwelling organisms, presenting a vector by which microplastic is introduced into the broader food web. Microplastic has been identified in fish and crabs that humans eat (Rochman et al. 2015, Watts et al. 2014). As discussed in the Introduction, the ingestion of microplastic may harm organisms through physical obstructions such as blockage or lacerations, or through exposure to harmful chemicals associated with the plastic.

Second, characterizing the concentration and composition of microplastic and nanoplastic in sediment is important for establishing a baseline by which the efficacy of potential management actions can be evaluated. For example, a federal microbead ban was enacted in 2015. This ban prohibits the use of microbeads in rinse-off bath and beauty products. The law bans production by July 2017 and sale of these products by July 2018. Evaluating sediments for microplastic over time can help us learn the efficacy of these types of management actions.

Third, identifying potential hotspots in the Bay may help us to evaluate the pathways by which microplastic is entering the Bay or the means by which microplastic is being generated *in situ*. The conceptual model describing how microplastic is hypothesized to enter the Bay (see Section 1; Sutton and Sedlak 2017) emphasizes two primary pathways by which microplastic is introduced into the Bay, urban stormwater runoff from creeks and rivers and treated effluent from Bay Area wastewater treatment plants. Sediment sampling will occur at strategically selected locations throughout the Bay in an effort to begin to characterize the baseline concentrations, compositions, and the potential contributions from these major pathways.

Lastly, these data will inform development of a model of the transport of microplastic in the Bay and out the Golden Gate. Microplastic has varying buoyancy depending on its chemical composition, as well as other factors including particle shape or type and the formation of biofilm coatings. Through comparison of particles present in sediment and surface water, it may be possible to qualitatively evaluate assumptions about particle settling essential to model the transport of microplastic. Model

development is intended to improve our understanding of the flow of microplastic within the Bay as well as the Sanctuaries, and to evaluate the efficacy of potential management actions to reduce pollution.

Sediment sample collection will be coordinated with small fish sample collection and will occur in the summer of 2017. The effect of seasonality is not being investigated for sediment or small fish.

4.2 Relevant Management Questions

This element, monitoring microplastic in sediment, addresses the following four questions:

- MQ1. How much microplastic pollution is there in the Bay?
- MQ3. What are the sources, pathways, loadings and processes leading to microplastic in the Bay?
- MQ4. Have concentrations of microplastic in the Bay increased or decreased?
- MQ5. Which management actions may be effective in reducing microplastic pollution?

MQ1 is most directly answered through this quantification of sediment concentrations of microplastic at locations representative of a variety of habitats within San Francisco Bay. The study will identify particle size, type (e.g. fragment, fiber, etc.) and composition (e.g., polyethylene, polypropylene, etc.). This information will be important as a first step in beginning to determine the magnitude of microplastic pollution in Bay sediment. In addition, this element may shed light on the sources, pathways, and processes (MQ3) through an evaluation of spatial patterns and characterization of particles (i.e., margin vs ambient concentrations and type of plastic in ambient sediments compared to those in sediments near urban creeks or wastewater treatment facilities). Sediment sampling locations were targeted to evaluate potential influence from urban runoff and wastewater treatment plant discharges relative to ambient Bay conditions.

MQ4, which addresses trends, will necessitate future sampling of sediments. This project will provide a baseline to which future monitoring efforts can be compared in years to come. A long-term goal is for temporal trends to be used to assess which management actions have the impact intended (MQ5). In addition, possible solutions for reducing concentrations of microplastic in the Bay may be informed by the particle types and sizes observed in sediment, where such information leads to identification of likely sources. For example, if a major portion of the microplastic particles identified in sediment are microbeads, then it is possible that the federal ban on microbead-containing rinse-off personal care products may address the issue. However, it is anticipated that a variety of microplastic types will be identified (e.g., fibers, film, fragments), which may be addressed through a myriad of options including educational outreach, bans on single-use or packaging materials, targeted trash management, etc.

These management questions present overarching goals for the RMP microplastic program. We have designed the sampling plan for sediment for this project based on specific questions that are articulated in the following section. Further discuss of how the results will be used to evaluate these hypotheses is discussed in Section 9.7.

4.3 Site Selection

The selection of sites to sample for sediments is driven by the following questions:

- Is the composition (e.g., particle type, polymer type) of microplastic and nanoplastic near pollution pathways different than that found in background Bay sites distant from pathways?
- Are microplastic concentrations and types in sediment correlated with microplastic concentrations observed in small fish collected from the same area?

Table 4.1 includes a summary of the location and number of sediment sites. Maps of the sediment sampling sites are shown in Section 8. Further details on the exact sampling locations, target site coordinates, and rationale for site selection are provided in Appendix B-2. Section 9.7 Treatment of Data discusses the methods by which these data will be evaluated.

Margin, Ambient Bay, and Reference Sites

As shown on Table B 2.1 in Appendix B, the sediment sites were selected to characterize microplastic concentrations near possible pathways in the nearshore "margins" of the Bay, in open or "ambient" portions of the Bay, and in a reference area. Most of the sites are focused in the Bay margins because the margins are closely linked to potential conduits of microplastic such as stormwater runoff from urban creeks and shallow wastewater discharges. As shown on Table B 2.1, we have classified the sites into one of four types: proximity to urban creeks or sloughs, proximity to wastewater treatment plant discharges, "background" sites that have no known pathways in the vicinity, and reference sites in Tomales Bay. We will evaluate the morphology and chemical composition of the microplastic identified at each of the site types to determine whether there are unique properties. As described in Section 9.7, it is likely that we will use principal component analysis to do so. Depending on the result, additional statistical analyses may be employed.

The RMP routinely monitors contaminants in surface sediments (top 5 cm) collected at sites at depths greater than 1 m below mean lower low water (referred to as the "open Bay" or ambient Bay). Specific sites are selected based on a Generalized Random Tessellation-Stratified design (GRTS) used by EPA's Environmental Monitoring and Assessment Program (EMAP). As shown on Table B-4.1 in Appendix B-4 and Figure 8.1, in 2014 as part of the RMP Annual Status and Trends cruise, 10 ambient Bay sediment samples were collected and archived for microplastic analyses from the Central Bay, South Bay, and Lower South Bay embayments. Although the samples collected were based on a randomized GRTS design, the 10 samples selected for microplastic were targeted and hence are not random. These 10 archived samples have been sent to the University of Toronto for microplastic analyses in 2017.

In addition to ambient Bay samples, recently, the RMP has devoted resources to characterizing sediment in the "Bay margins," or locations ranging from depths less than 1 m below mean lower low water to the unvegetated shoreline (roughly mean high water). These areas generally include mudflats and adjacent areas. Bay margins are productive and highly-utilized by both humans and wildlife, but have not previously been monitored by the RMP, in part due to logistical considerations (sampling by a boat with

about a 1 m draft). Analysis of margins contaminant concentrations in the RMP Margins Conceptual Model Report (Jones et al. 2012) suggested higher and more variable chemical contaminant concentrations in the Bay margins, but much of the historical monitoring was not randomly distributed and instead focused on known chemical contaminant hotspots of management interest.

The RMP began conducting a pilot effort to sample sediment in the Central Bay margins in 2015. Forty Bay margins sites were targeted for sediment sampling, of which 10 sites were targeted for sampling for microplastic (see Table B-4.1 in the Appendix B-2). The pilot study was focused on the Central Bay, which is a highly urbanized area with a large number of known trash hot spots. The microplastic sites were not randomly chosen but rather selected based on site characteristics that might suggest a higher microplastic concentration (e.g., vicinity of known trash hot spots, etc.). Additional information regarding the collection of samples and sites can be found in the 2015 Annual Monitoring Results report (see http://www.sfei.org/documents/2015-annual-monitoring-report).

RMP margin sampling will continue in 2017 with sediment collection in the South and Lower South Bays. At the writing of this report, RMP staff are determining the site locations for the larger RMP margin study. The Moore Microplastic project will sample 16 sites out of the 40 RMP margin sites targeted for summer 2017. The approximate locations of these 16 sites are identified in Table 4.1 and Figures 8.1-6, and described in greater detail in Appendix Table B-4.1. Approximately half of these sites (~8) will be targeted for nanoparticle analyses due to the relatively high volume of sediment needed for nanoplastic analyses and the short tidal windows available for sampling that necessitate fewer sediment sample grabs. In addition to the RMP margin sediment cruise, this project will collect sediment from 8 margin sites in the North Bay (5 sites in San Pablo and 3 sites in Suisun Bays). For the North Bay sites, GRTS sampling design will be used to select sites in these embayments; however, a targeted subset of these GRTS sites will be used for microplastic.

Although the margin sediment sites were identified based on a random pull as part of the GRTS design, the microplastic sites were selected based on proximity to sources such as urban creeks or wastewater facilities and, therefore, are not random. As such, they cannot be used to make strict spatial inferences for the Bay as a whole.

Lastly, three sediment margin sites are located in Tomales Bay, which is part of the Point Reyes National Seashore. This area is relatively undeveloped and has been selected as a reference site. The location of these sites is presented on Figure 8.7.

Correlation of Sediment Microplastic with Small Fish Microplastic

As shown on Table B-2.1 in Appendix B-2, at eight of the margin sediment sites, small fish will be collected. In each embayment, 20 small prey fish will be collected at each of two margin sediment sites. Two different species of fish will be targeted at each site (10 of each species). Further discussion of the selection of prey fish is presented in Section 5. Microplastic in co-located sediment and water will be evaluated to assess the fate and uptake of microplastic.

4.4 Field Sample Collection Methods

Microplastic and Nanoplastic Samples

Sediment samples will be collected shipboard using a Van Veen sediment grab. Samples will be directly scooped into sampling containers. A detailed description of the sampling methodology is included in the Appendix B-2. A subsample of 500 mL will be collected for the microplastic analyses, a subsample of 800 mL will be collected for nanoparticle analyses, and another subsample of 800 mL will be archived. Both microplastic and nanoparticle samples will be collected into glass jars, stored and shipped on ice to University of Toronto for microplastic analyses, and University of Michigan for nanoplastic analyses, respectively. All glass sample jars will be purchased pre-cleaned from VWR or a comparable laboratory equipment supplier. Field measurements such as salinity and depth to bottom sediments will be noted on the field collection sheets discussed in Appendix B-2.

Quality Assurance / Quality Control Samples

Field blanks will be taken to assess the potential for the introduction of microplastics during sample collection and transport; field duplicates will be collected to assess the variation in the sample matrix. To assure that sampling tools are clean, field equipment will be rinsed with filtered DI water three times. A blank will be collected by rinsing the sampling tools in the field with filtered DI water and the resulting rinseate will be collected into a pre-cleaned glass sample bottle. The field blank will be collected prior to collecting the field sample in the field.

Field duplicates will be collected at three sites; at these sites no short term archive will be collected. Four 1-L sample jars will be filled (instead of only two sample jars). two jars will be sent to each lab for analysis. Field duplicate samples will be analyzed by analyzing samples from separate jars. If field duplicates are significantly different for the microplastic analysis, then two lab duplicate samples will be analyzed from a composite of the remaining sediment from both jars. A description of the processing for microplastic analyses is included in Appendix B-2.

4.5 Laboratory Analyses

Microplastic analyses (20 micron and larger)

Sediment samples will be processed and analyzed for microplastic by the Rochman Lab at University of Toronto using methods modified by Corcoran et al. (2015). Microplastic particles will be characterized from 20 microns up to approximately 5 mm. A brief summary of the laboratory method is presented below.

The sediment samples will be dried at 90 °C and added to a sodium polytungstate (SPT) density solution of 1.5 g cm^{-3}. The mixture will be magnetically stirred for 2 minutes and then transferred to a glass separation funnel to settle overnight. The non-buoyant material will be drained into a beaker, followed by draining of the buoyant material (low density) into a separate beaker. The low density material will be rinsed thoroughly with distilled water and filtered onto a polycarbonate filter for analysis. If filter

samples contain too much organic material for spectroscopy, samples will be resuspended in 4N KOH, digested. Like the microplastic analysis for water samples described in Section 3.5, digested samples will be filtered through a 10-micron polycarbonate filter and sealed to prevent cross-contamination. Filters will be analyzed via Raman spectroscopy using Particle Finding software that scans each suspect particle across the filter. Microplastics will be characterized by shape, size and type via Raman spectroscopy. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, chemical composition, and particle type will be reported. Photographs of the particles will also be included in the data package. Further discussion of laboratory methods is presented in the Appendix C.

Particles greater than 5 mm will be processed in a similar manner and identified using Raman spectroscopy as described above and enumerated.

Nanoparticle analyses (< 1 micron)

Sediment samples will be analyzed for nanoparticles by the Duhaime/ Banaszak Lab at the University of Michigan. Separation of nanoparticle plastic using a SPT density gradient will also be employed. The particle-containing solutions will be directly adsorbed or spin-coated onto freshly prepared clean substrates (i.e., mica, graphite, MoS_2 and chemically modified forms thereof). If sufficient particles are not present for this approach, Langmuir-Blodgett approaches will be pursued to form monolayer films for study, and/or filtration approaches may be used. AFM-IR will be employed to characterize the particle size, morphology, and chemical composition of the materials. If fields of particles are imaged, statistical information will be generated. Images of particles will be provided. Further discussion of methods is presented in Appendix C.

Quality Assurance / Quality Control

As noted in Section 4.5, laboratory QA/QC will involve best practices (i.e., wearing cotton clothing, filtering in a clean space and not allowing samples to be exposed to the air when possible, using clean glassware and ultrapure water) for reducing procedural contamination, and will use laboratory and field blanks to account for any procedural contamination. In the laboratory, one laboratory blank sample will be run each day that the lab performs extractions. For batches of more than 10, there will be one lab blank per 10 samples. Lab blank samples will be filtered DI water that has gone through the same procedure for extraction and analyses as the real samples, i.e., digested in KOH, filtered and analyzed via Raman spectroscopy. Microplastic in the field and lab blank samples will be characterized and microplastic of the same type and morphology will be subtracted (blank corrected) from the field samples to account for procedural contamination. The results of the blanks will be reported for all analyses.

Table 4.1 Total number of microplastic and nanoplastic samples collected or to be collected at San Francisco Bay ambient and margin sediment sites

Location	Subregion	Sediment Type	# Sediment Samples	# Sediment Blanks	# Sediment Field Duplicates	# Nano Samples	# Nano Blanks	#Nano Field Duplicates	Short term archives
	Mowry	Margin	1			1			1
	Near Palo Alto WWTP	Margin	1			1			1
	Hooks Point	Margin	1			1			1
	Coyote Creek	Margin	1		1	1		1	1
	Shoreline	Margin	1			1			1
	Jagel Slough	Margin	1			1			1
	Don Edwards	Margin	1			1			1
	San Francisquito	Margin	1	1		1	1		1
Lower South Bay	Main stem of LSB	Ambient	2			2			
	South of SFO	Margin	1			1			1
	San Mateo Creek (Seal Slough)	Margin	1		1	1		1	1
	Bair Island	Margin	1			1			1
	Redwood Creek	Margin	1			1			1
	Ravenswood Slough	Margin	1			1			1
	Oyster Bay	Margin	1	1		1			1
	Near Oro Loma	Margin	1			1			1
	Eden Landing	Margin	1			1			1
South Bay	Main Bay	Ambient	4						
	Richardson Bay, Marin	Margin	1						
	Richmond/ Albany	Margin	2						
	Emeryville	Margin	1						
	SF McCovey Cove	Margin	1						
Central Bay	San Leandro Bay/ Alameda Coast Guard Island	Margin	3						

Table 4.1 Total number of microplastic and nanoplastic samples collected or to be collected at San Francisco Bay ambient and margin sediment sites									
	Oyster Point	Margin	1						
	Crab cove, Alameda	Margin	1						
	Main Bay	Ambient	4						
	San Pablo Bay - China Camp	Margin	1	1		1	1		1
	San Pablo Bay- Petaluma River	Margin	1		1	1		1	1
	San Pablo Bay- Sonoma Creek	Margin	1			1			1
	San Pablo Bay- Napa	Margin	1			1			1
	San Pablo Bay- Hercules	Margin	1			1			1
	Suisun Bay-Contra Costa WWTP	Margin	1			1			1
	Suisun Bay -Montezuma slough	Margin	1			1			1
North Bay	Suisun Bay- Point Edith Wildlife	Margin	1			1			1
	South Bay	Margin	1			1			1
	Walker Creek	Margin	1			1			1
Tomales Bay	Marshall	Margin	1	1		1	1		1
TOTAL			**54**			**26**			**27**
			TOTAL SAMPLES for ANALYSES: 80						

1—Microplastic samples are to be collected in a 500 mL container; nanoplastic samples in a 1 L container; and short term archive samples in a 1 L container.

5 San Francisco Bay Prey Fish

5.1 Objective

The goal of this element is to quantify the abundance of microplastic in prey fish. The prior microplastic screening study conducted in 2015 identified 52 particles in nine small prey fish from the Bay. This is the only study that has evaluated microplastic in Bay organisms. Based on the paucity of data, the Microplastic Monitoring and Science Strategy (Sutton and Sedlak 2017) and attendees of the 2016 RMP workshop placed a high priority on the characterization of microplastics in biota, particularly fish.

A phased approach for evaluating prey and sport fish will be undertaken consistent with the Microplastic Strategy (Sutton and Sedlak 2017). In the first phase, prey fish will be monitored to assess the health of an important food source for wildlife as well as to evaluate the potential for microplastics to bioaccumulate in wildlife or humans. The RMP will monitor sport fish for chemical contaminants in 2019 as part of a five-year monitoring cycle of sport fish conducted by the RMP Status and Trends program. If prey fish are found to have significant concentrations of microplastic, a second phase consisting of monitoring of sport fish for microplastic will be proposed to the RMP and external funders.

Fish sampling will be co-located with the sediment sampling and will occur in the summer of 2017. The effect of seasonality is not being investigated for sediment or prey fish. Section 9.7 discusses the methods by which these data will be evaluated.

5.2 Relevant Management Questions

Monitoring microplastic in prey fish addresses the following management questions:

- MQ1 How much microplastic pollution is there in the Bay?
- MQ2 What are the health risks?
- MQ3 What are the sources, pathways, loadings and processes leading to microplastics in the Bay?
- MQ4 Have concentrations of microplastic in the Bay increased or decreased?
- MQ5 Which management actions may be effective in reducing microplastic pollution?

This element of the study will help to quantify levels of microplastic in San Francisco Bay prey fish (MQ1). This is an important question for evaluating risk to prey fish, as well as evaluating the potential risk to higher trophic level organisms such as sport fish and humans (MQ2). Intrinsic to the question of whether microplastic is being ingested is whether chemical contaminants that are present in microplastic, such as plasticizers, dyes, and flame retardants, or chemicals that are attracted to plastics, such as polybrominated diphenyl ethers, are bioaccumulating as a result of plastic ingestion (Farrell and

Nelson 2013; Rochman et al. 2014a; Setala et al 2014). Moreover, if microplastic particles are small enough, the plastic itself may bioaccumulate if it translocates across the gut and into the tissues of an animal (Browne et al., 2008). In the screening study conducted in 2015, nine prey fish were caught as bycatch and were subsequently analyzed for microplastic. Fifty percent of the microplastic particles were classified as fragments, while 33% were classified as fibers (Sutton et al. 2016). Interestingly, in the only other California study to characterize microplastic in fish, fibers were the most common particles detected in fish (Rochman et al. 2015). Characterization of the type of microplastic (e.g., fragment or fiber, etc.) and chemical composition (e.g., polyethylene, polystyrene, etc.) will help in determining the sources of microplastic (MQ3) and possible actions that can be taken to mitigate impacts (MQ5).

In addition, prey fish monitoring may help to identify areas of particular concern (high exposure) at a regional and local scale and provide a foundation for tracking interannual trends. This project will provide a baseline from which future sampling efforts can be compared (MQ4). This will also be important in assessing which management actions may be most effective (MQ5).

Lastly, it will be important to identify the type of microplastic that are consumed by fish. Fish may exhibit preferences for particle size or chemical composition; different species may have differing preferences based on ecological niche, foraging patterns, and diet preferences. If there are preferential feeding patterns, this may help to inform mitigation measures (MQ5).

These management questions present overarching goals for the RMP microplastic program. We have designed the sampling plan for fish for this project based on specific questions that are articulated in Section 5.4. Further discuss of how the results will be used to evaluate these hypotheses is discussed in Section 9.7.

5.3 Target Fish Species

Prey fish are desirable for monitoring for several reasons. They are important prey for piscivorous Bay fish, birds, and marine mammals. They exhibit relatively high site fidelity, allowing for comparisons among embayments. They typically have short life spans, on the order of a year or two, so they provide a temporal snapshot of conditions in time. They provide an indication as to whether contaminants are bioavailable. Lastly, in some instances, a correlation can be made between concentrations of contaminants in sediment and concentrations in prey fish, as has been demonstrated for PCBs (Greenfield and Allen 2013).

This element focuses on three different species that have been successfully monitored in the past: Mississippi silverside, topsmelt, and anchovy. They represent three different ecological niches: Bay water that is influenced by freshwater input from urban creeks; Bay margins; and more open channel portions of the Bay, respectively. Mississippi silverside (*Menidia audens*) and topsmelt (*Atherinops affinis*) have been used extensively as a biosentinel species for monitoring the uptake of contaminants

such as mercury and PCBs in the Bay (Greenfield and Allen 2013; Greenfield et al. 2013; Greenfield and Jahn 2010; Melwani et al. 2012). Based on past RMP work, it is advantageous to target both silversides and topsmelt at all sites, as the salinity level may favor one species or the other. For example, Mississippi silversides are rarely found in Central Bay, whereas topsmelt are frequently observed there (Davis et al. 2016). In addition to topsmelt and silverside identified in the margins, we are proposing to monitor a more pelagic prey fish, the northern anchovy (*Engraulis mordax*).

Mississippi Silverside (Menidia audens)

Mississippi silverside, a non-native species, was introduced into Bay Area lakes in the late 1960s and spread rapidly across the watersheds and into the Bay (Moyle 2002). Silverside typically reside along the shallow Bay margins near marshes and mouths of creeks. They are primarily considered a freshwater species that is rarely found in the offshore areas of San Francisco Bay (Davis et al. 2016). They are believed to have high site fidelity with an estimated home range of 1 to 10 km (Melwani et al. 2012). Silverside prey on small benthic invertebrates such as copepods and cladocerans, epibenthic crustaceans (corophiid amphipods), insects, and small pelagic invertebrates (Melwani et al. 2012).

Topsmelt (Atherinops affinis)

In contrast to silverside, topsmelt are native to the Bay and prefer saltwater, residing in shallow bays, sloughs, and embayments (Davis et al. 2016). Topsmelt have a slighter wider home range compared to silversides, moving into deeper waters during low tides (Davis et al. 2016). Topsmelt are abundant in the shallow margins of the Bay from May to September and then migrate to deeper waters in the winter months. The diet of topsmelt is very similar to silverside; they forage on benthic and pelagic plants and invertebrates, diatoms, midge larvae and amphipods (Melwani et al. 2012).

Northern Anchovy (Engraulis mordax)

Northern anchovy is the most abundant fish species in San Francisco Bay (SFEP 1992). They are critical forage fish for higher trophic predator species such as Chinook salmon, California halibut, and leopard sharks (Goals Project 2000). They are found throughout San Francisco Bay in more pelagic conditions in comparison to silverside, although it appears that they have a slight preference for wastewater treatment plant outfalls (SFEP 1992). Anchovy typically migrate into the Bay in the spring to feed and head out to the ocean in the winter, although some juvenile fish overwinter in the Bay. The diet of anchovy is diverse and consists of plankton, zooplankton, crustaceans, and fish eggs, and larvae (Goals Project 2000; SFEP 1992). They typically forage on plankton throughout the water column, in contrast to the other species that focus more on the benthic invertebrates.

5.4 Site Selection

Fish site selection was driven by four factors. First, there was a strong desire to co-locate the existing margin sediment sampling with the fish sampling to evaluate whether sediments may be a source of microplastic to the food web and to determine whether there were regional hotspots. Second, there was a strong interest in placing some of the fish sites near pathways (e.g, stormwater or wastewater

outfalls) to ascertain the influence of pathways on uptake of microplastic. Third, where possible, fish sites are located in close proximity to a surface water site. Lastly, all sites were reviewed by Moss Landing Marine Lab staff to assess the probability that fish would be available at the targeted site based upon the staff's knowledge of these sites. It will be important to assess the morphology and chemical composition of the microplastic in fish, in part to see whether qualitative inferences can be made about potential sources such as sediment, surface water, stormwater runoff or effluent. Further discussion of the treatment of the data is presented in Section 9.7.

Eight sites were selected to monitor small prey fish (Table 5.1). As described above, all sites are co-located with sediment sampling sites, and many are in close proximity to urban creeks or wastewater effluent outfalls. As shown in Table 5.1, at several of these sites, stormwater, wastewater, and Bay water samples will be collected. Figures showing site locations are presented in Section 8. Two sites are located in Tomales Bay, a reference site located adjacent to a Point Reyes National Seashore.

5.5 Field Sample Collection Methods

At each site, 20 individual small prey fish will be collected for microplastic analyses. Because anchovy are observed throughout the Bay, 10 individuals of this species will be targeted for collection at each of the eight sites. Another 10 prey fish - either topsmelt or Mississippi silverside - will be collected at each site, depending on site characteristics and the ability to catch the targeted prey fish. At two sites (Tomales and South Bay), 10 additional fish will be collected for nanoplastic analyses. Any additional fish that are caught at the sites will be archived for potential future analyses.

Small prey fish (50 to 120 mm) will be collected using an otter trawl, cast net, or beach seine depending on the location and target fish. All fish samples will be individually wrapped in foil and placed immediately on wet ice on the boat and then on dry ice at the end of each day, before being placed in a -20 degree C freezer at the laboratory. A field collection form will be used to document the collection and to obtain field parameters (see Section 7 in Appendix B-3). Additional information regarding field collection procedures is presented in Appendix B-3.

To date, a standardized method for collecting field blanks and duplicates has not been developed. A field blank for fish will not be collected. Because 10 fish from each species will be collected at each site, we will have information on the variation among fish at any given site; therefore, a separate field duplicate will not be collected.

5.6 Laboratory Analyses

Microplastic analyses (20 micron and larger)

Fish will be thawed and dissected to remove gut and gut contents. Microplastic particles 20 microns and larger will be characterized. Fish guts will be extracted at room temperature using 4N KOH solution for up to 14 days (Rochman et al. 2015; Dehaut et al. 2016). After digestion, the sample will be filtered

through a 10-micron filter and analyzed using Raman spectroscopy. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, chemical composition, and particle type will be reported. Photographs of the particles will also be included in the data package. Further discussion of methods is presented in the Appendix C.

Nanoparticle analyses (< 1 micron)

The University of Michigan will develop a method for evaluating nanoplastic (<50 nm) in fish. Nanoplastic analysis will be performed on a limited subset of fish from one site in the Lower South Bay (Guadalupe) and one site in the reference area (Tomales southern portion of the Bay).

Quality Assurance / Quality Control

In general, laboratory QA/QC will involve best practices (i.e., wearing cotton clothing, filtering in a clean space and not allowing samples to be exposed to the air when possible, using clean glassware and ultrapure water) for reducing procedural contamination, and will use laboratory blanks to account for any procedural contamination. In the laboratory, one laboratory blank sample will be run each day the lab that performs extractions. For batches of more than 10, there will be one lab blank per 10 samples. Lab blank samples will be filtered DI water that has gone through the same procedure for extraction and analyses as the real samples, i.e., digested in KOH, filtered and analyzed via Raman spectroscopy. As noted above, a field blank for fish will not be collected, nor will a separate field duplicate. Microplastic in the lab blank samples will be characterized and microplastic of the same type and morphology will be subtracted (blank corrected) from the field samples to account for procedural contamination. The results of the blanks will be reported for all analyses.

Table 5.1 Prey fish samples sites and correlation with other samples collected					
Site[1]	**Location**	**Sediment site**	**Stormwater site**	**Wastewater site**	**Adjacent surface water site**
Tomales Bay (Reference)	Tomales South	Yes			
Tomales Bay (Reference)	Tomales North - near Walker Creek	Yes			
Central Bay	Just slightly northwest of Bay Bridge/IKEA (CB15)	Near CB-15	AC-2017-Line12A	EBMUD	Yes
Central Bay	San Leandro Bay - NE near East Creek Slough (CB32)	Near CB-32	AC-2017- Line12F		Yes
Lower South Bay	Near Hooks Point	Yes		Palo Alto	Yes
Lower South Bay	Near Guadalupe River	Yes	Guadalupe River (upstream)	San Jose	Yes
North Bay	San Pablo Bay near Petaluma River	Yes			
North Bay	San Pablo Bay near China Camp	Yes			

1 -- At each site, 10 anchovy and 10 silverside or topsmelt will be collected (20 individual fish). Any bycatch or excess fish will be wrapped in foil and archived. At the Lower South Bay (Guadalupe) and the Tomales Bay (Southern portion of the Bay), 10 additional prey fish (silverside or topsmelt) will be collected for preliminary nanoplastic analyses.

6 Pathways: Wastewater Effluent

6.1 Objective

The goal of this element is to characterize microplastic in treated wastewater. In 2015, a limited screening study was undertaken to evaluate microplastic in effluent from eight wastewater treatment facilities, representing approximately 60% of the effluent flow into San Francisco Bay (Sutton et al. 2016). The facilities were geographically distributed, varied in capacity from 2.3 million gallons per day (MGD) to 310 MGD, and employed a range of secondary and tertiary treatments. Microparticles were identified in all of the effluent samples; 80% of the particles were characterized as fibers, followed by fragments at 17%. The remaining portion was characterized as film or foam. Visual identification was used to characterize the particles; spectroscopic polymer identification was not available. Therefore, it is possible a portion of the effluent-derived particles were not plastic. Additional work suggests that a variety of nonplastic materials are present in Bay Area effluent (Dyachenko et al. 2016).

On average, approximately 0.33 particles per gallon were discharged to the Bay. The levels in effluent are higher than those found in effluent from the Midwestern and Northeastern U.S. using identical methods (Mason et al. 2016). This difference may be in part attributed to the water conservation efforts employed at the time in California to mitigate the impacts of a drought. Nevertheless, the presence of fibers is consistent with other studies that have identified this particle type as common in effluent (Browne et al. 2011; Mason et al. 2016). A recent study of outdoor garments estimated that between 0 to 2 grams of microfibers (representing 0.3% of the mass of the garment) may be shed during washing (Hartline et al. 2016). In a separate study, it was estimated that a single garment can release more than 1,900 microfibers per washing cycle (Browne et al. 2011).

As noted above, the 2015 Bay screening study employed visual inspection to identify microplastic; no secondary confirmation using spectroscopy was conducted. Although fibers are more reliably identified through visual inspection as plastic than fragments (Lenz et al. 2015), there is concern that not all of the fibers identified in the screening study were plastic. Another study has indicated that some of the microfibers ingested by invertebrates were not actually plastic but rather an artificial cellulose material, viscose or rayon (Remy et al. 2015). Follow-up work by the Bay Area Clean Water Association (BACWA) laboratories found that fats, oils, and natural fibers like cotton could persist after sample processing using the NOAA method (Masura et al. 2015; Dyachenko et al. 2016). To address this issue, this project will use Raman spectroscopy to chemically confirm the composition of the particles. Section 9.7 discusses the methods by which this data will be evaluated.

This element will occur in Fall 2017.

6.2 Relevant Management Questions

The Microplastic Monitoring and Science Strategy has articulated five management questions for San Francisco Bay (Sutton and Sedlak 2017). This element, monitoring microplastic in wastewater, addresses the following three questions:

- MQ1. How much microplastic pollution is there in the Bay?
- MQ3. What are the sources, pathways, loadings and processes leading to microplastic in the Bay?
- MQ5. Which management actions may be effective in reducing microplastic pollution?

This project aims to determine microplastic pollution concentrations in samples taken directly from eight wastewater treatment facilities that discharge into San Francisco Bay. The data will be used to answer MQ3. Characterizing effluent discharges will provide information essential to estimate the loadings derived from this critical pathway. Because there are limited sources of down-the-drain plastic pollution, this characterization will also provide better information on the relative importance of different sources, including products containing microbeads and fibers derived from synthetic textiles. This information, combined with targeted Bay sampling, will improve estimates of Bay pollution informed by modeling activities (MQ1). Information specific to these sources and the wastewater pathway are expected to inform discussions of management actions, including actions that residents in service areas can take to reduce their personal contributions to plastic pollution (MQ5).

These management questions present overarching goals for the RMP microplastic program. We have designed the sampling plan for wastewater for this project based on specific questions that are articulated in the following section. Further discuss of how the results will be used to evaluate these hypotheses is discussed in Section 9.7.

6.3 Site Selection

Eight facilities will be targeted that have varying treatment trains (e.g., secondary vs. advanced). Although the 2015 screening study did not show a significant variation in microplastic levels based on treatment (Sutton et al. 2016), it is noteworthy that one of the largest facilities with advanced treatment contained only microfibers, suggesting that there may be successful methods for removing microplastic fragments. In addition to evaluating a variety of treatment options, there is a preference for larger facilities (greater than 10 MGD) to better characterize the load of microplastic into San Francisco Bay. The size of the population served has been shown to be positively correlated with particles discharged (Mason et al. 2016).

At the writing of this Sample and Analysis Plan, seven facilities have indicated an interest in participating in this study, as shown on Table 6.1. We are currently reaching out to one more facility. There are approximately 35 wastewater facilities in the Bay Area; four of these are identified as discharging into the Pacific Ocean.

Modeling needs

To better understand the fate and transport of microplastic within the Bay and the exchange with the adjacent Pacific Ocean, it will be important to develop a model of microplastic transport. Microplastic transport modeling will be carried out with particle tracking models, predicting trajectories of virtual microplastic particles as they are transported within the Bay and out into the coastal ocean. Key inputs for the modeling effort will be estimates of discharge of microplastics from stormwater and wastewater pathways. This wastewater monitoring effort supplies essential inputs for the particle transport model.

6.4 Field Sample Collection Methods

Effluent from eight wastewater treatment facilities will be collected over a 24-hour period to obtain a more representative sample relative to the 2015 RMP study, which sampled over 2 hours during peak flow (Sutton et al. 2016). Additional work by BACWA has confirmed that collection of 24-hour composites is possible and preferable (Dyanchenko et al. 2016). Two samples will be collected from each facility; samples will be collected Tuesday through Friday, to avoid the potential influence of different consumer behaviors over the weekend.

Wastewater flow can vary throughout the day and throughout the seasons. To mitigate the impacts from increased flows during wet weather, the wastewater samples will be collected in the dry season.

Final treated effluent will be collected from a sampling port prior to the effluent being discharged. The effluent will be passed through 8-in. diameter stacked Tyler sieves with 355 micron and 125 micron stainless steel mesh. The 125 micron mesh has been found to be particularly useful for retention of microbeads discharged to the sewer via use of personal care products (Napper et al. 2015; Carr et al. 2016). To prevent the sieves from clogging, we may include two additional sieves (5 mm and 1 mm) per the method developed by BACWA (Dyanchenko et al. 2016).

Rate of flow at the point of collection must be measured to calculate of number of particles per volume of treated wastewater. Previous, 2-hour monitoring efforts involved measurement of flow before and after each sample (Sutton et al. 2016). However, given the length of time for this sampling effort (24 hours), it may be necessary to measure flow more frequently, or to use a flow monitor employed at the sampling point. In addition, the 24-hour discharge flow rate for the plant on the day of sampling will be obtained, to allow for estimation of the number of particles discharged per day. Sieves will be processed in the laboratory and, if needed, preserved with alcohol prior to shipping. Microplastic collected in the sieves will be gently washed using distilled water into glass sample bottles prior to shipping to University of Toronto for analyses.

A field collection form will be used to document the collection and to obtain field parameters (see Section 7 in Appendix B-4).

Quality Assurance / Quality Control Samples

A field blank will be collected at one site by setting up a sieve set in the nearby vicinity of the field sampling sieve set. The field blank sieves will remain uncovered for the duration of the 24-hour sampling event to assess contamination from the deposition of airborne microparticles during the 24-hour sampling event. A field duplicate will be collected at one site by using a Y splitter on the sampling port. Effluent will be diverted to two stack sieves and a 24-hour composite will be analyzed.

6.5 Laboratory Analyses

Microplastic analyses (125 micron and greater)

The extraction of microplastic from wastewater will be undertaken at room temperature using 4N KOH solution for up to 14 days (Dehaut et al. 2016). After digestion, the sample is filtered through a 10 micron polycarbonate filter and analyzed using Raman spectroscopy. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, chemical composition, and particle type will be reported. Photographs of the particles will also be included in the data package. Further discussion of methods is presented in the Appendix C. Wastewater samples will not be analyzed for nanoplastic.

Quality Assurance / Quality Control

A goal of this project is to develop more standardized methods and techniques. NOAA has developed a method for analyzing for microplastic (Masura et al. 2015); however, several shortfalls have been identified with this method, particularly for wastewater samples (Dyachenko et al. 2016), and the field is continuing to improve methods for analyses of microplastic (see for example Dehaut et al. 2016; Corcoran et al. 2015). The laboratory processing methods and laboratory analyses that are being employed as part of this project have been shown to perform well (Dehaut et al. 2016).

In general, laboratory QA/QC will involve best practices (i.e., wearing cotton clothing, filtering in a clean space and not allowing samples to be exposed to the air when possible, using clean glassware and ultrapure water) for reducing procedural contamination, and will use laboratory and field blanks to account for any procedural contamination. In the laboratory, one laboratory blank sample will be run each day the lab that performs extractions. For batches of more than 10, there will be one lab blank per 10 samples. Lab blank samples will be filtered DI water that has gone through the same procedure for extraction and analyses as the real samples, i.e., digested in KOH, filtered and analyzed via Raman spectroscopy. Microplastics in the field and lab blank samples will be characterized and microplastics of the same type and morphology will be subtracted (blank corrected) from the field samples to account for procedural contamination. The results of the blanks will be reported for all analyses.

Wastewater Loadings Estimates

Using the daily flow, we will calculate loads of microplastic from each of the facilities. These first order load estimates represent a starting point for characterizing possible loads from wastewater.

Table 6.1 Wastewater Treatment Facilities - Location, Characteristics and Alignment with Other Sites						
Site[1]	**Location**	**Treatment**	**Design Flow (MGD)**	**Sediment site[2]**	**Fish**	**Adjacent surface water site[2]**
North Bay	Central Contra Costa Sanitation District	Secondary	~50	Yes		
Central Bay	San Francisco Public Utilities Commission-Southeast	Secondary	86			
Central Bay	EBMUD	Secondary	~120			
South Bay	East Bay Dischargers Authority	Secondary	77			Yes
South Bay	Fairfield Suisun	Advanced				
Lower South Bay	Sunnyvale	Advanced	~30	Yes		Yes
Lower South Bay	Palo Alto	Advanced	39	Yes	Yes	Yes
Lower South Bay	San Jose Santa Clara	Advanced	167	Yes	Yes	Yes

1—The location of the field duplicate and field blank will be determined; however, given that only fibers were detected at San Jose Santa Clara facility, this facility will not be chosen as a duplicate site.

7 Pathways: Stormwater

7.1 Objective

Stormwater runoff is believed to be one of the primary pathways for plastic pollution to enter the Bay (Figure 2.1; Sutton and Sedlak 2017; BASMAA 2014a; EOA 2014). At present, there is no information on microplastics in Bay stormwater, a data gap noted in the prior RMP study (Sutton et al. 2016). There is limited research on trash (greater than 5 mm) in receiving waters entering San Francisco Bay (BASMAA 2016; BASMAA 2014a; EOA 2014). One objective of this project will be to examine microplastic in stormwater, adding substantially to extremely limited research worldwide characterizing this matrix. No standardized methods exist for collecting microplastic particles from stormwater. This project will develop and test new methods for collecting stormwater samples.

Stormwater sampling will be coupled with surface water, sediment, and fish samples that are taken near points of discharge, with the intention of better documenting plastic pollution levels entering San Francisco Bay. Section 9.7 discusses the methods by which these data will be evaluated.

While there is no information regarding the contribution of microplastic from stormwater to the Bay, trash monitoring studies have been conducted in local storm drains and demonstrate the ubiquity of larger plastic items within urban litter (e.g., BASMAA 2016; BASMAA 2014b; EOA 2014). Storm events likely play a major role in mobilizing macro- and microplastic derived from litter. A southern California study evaluating inputs from the Los Angeles River drainage to the coastal ocean near Long Beach found that concentrations of microplastic increased 7-fold following a storm, from 8 pieces per cubic meter to 56 pieces per cubic meter (Moore et al. 2005).

This element will occur in Winter 2017 through Spring 2018.

7.2 Relevant Management Questions

The Microplastic Monitoring and Science Strategy has articulated five management questions for the San Francisco Bay (Sutton and Sedlak 2017). This element, monitoring microplastic in stormwater, addresses the following three questions:

- MQ1. How much microplastic pollution is there in the Bay?
- MQ3. What are the sources, pathways, loadings and processes leading to microplastic in the Bay?
- MQ5. Which management actions may be effective in reducing microplastic pollution?

This element will characterize microplastic pollution in samples collected from urban creeks or municipal drainage channels to provide information to answer MQ3. This element will characterize microplastic from a variety of watersheds. It is possible that different watersheds may have different sources of

microplastic. Microplastic samples will be analyzed for particle size, morphology (e.g., fragment, fiber, etc.) and composition (e.g., polyethylene, polypropylene, etc.), that will not only be used to determine concentration but may also identify possible sources which may influence management actions (MQ5). In addition, information on stormwater, combined with targeted Bay sampling, will improve estimates of Bay pollution informed by modeling activities (MQ1).

MQ4 addresses long-term temporal trends, with the specific goal of understanding the forces that lead to any identified trends, including changes in sources (e.g., urban/consumer use of plastic), implementation of management actions relating directly or indirectly to control of plastic or microplastic, and other, larger variables such as climate change and drought. This element is important as it will address a significant data gap.

Evaluation of potential sources of microplastic may aid in identifying management actions, helping to answer MQ5. As mentioned in the previous section, this study will help better understand how microplastic enters San Francisco Bay. This research will provide baseline data to estimate microplastic loading via these pathways. Eventually, pathway data can be compared to other possible pathways, including spills and illegal dumping as well as wind transport, and with the *in situ* Bay process of fragmentation of larger plastic debris to form microplastic.

These management questions present overarching goals for the RMP microplastic program. We have designed the sampling plan for stormwater for this project based on specific questions that are articulated in the following section. Further discuss of how the results will be used to evaluate these hypotheses is discussed in Section 9.7.

7.3 Site Selection

The selection of sites to sample stormwater is driven by the following questions:

- Are concentrations from urban sites higher than rural, open and undeveloped spaces?
- Does the size of the watershed influence the levels of microplastics observed?
- Do sites that have been identified as trash hot spots have higher concentrations of microplastic?

The project aims to monitor up to 15 sites depending on weather conditions and ability to collaborate with existing RMP efforts. Approximately 37 possible stormwater monitoring sites have been reviewed and prioritized based on their importance and significance in understanding microplastic in San Francisco Bay (Table 7.1). The sites were prioritized based on drainage area, geographical distribution throughout the Bay, and proximity to known trash hotspots. At several locations, multiple stormwater samples are targeted along an urban creek; higher priority is given to those sites located farthest downstream. Higher priority was also given to large urban streams and watersheds. In addition, sites that correlate with Bay fish and sediment sampling (e.g., San Leandro Bay and Lower South Bay Guadalupe sites) were also given higher priority. Lastly, a priority was placed on obtaining samples from a variety of watersheds - rural, commercial, residential, etc.

Of the 37 sites, 34 sites are part of the RMP's Small Tributary Loading Program. Significant effort is involved in stormwater sampling including tracking storm systems and mobilizing field teams to work in challenging storm conditions over long periods of time. As a result, this element of the project will leverage the existing RMP stormwater sampling that is already occurring.

The focus of the RMP stormwater monitoring is estimating loads within local watersheds for several legacy contaminants. While RMP stormwater sites have been selected largely to identify possible industrial sources of mercury and PCBs, the attributes of many of these sites make them desirable for sampling microplastic. Therefore, this project will augment existing efforts to the extent possible. Samples for the Small Tributary Loading Program are collected during storms where ~0.50 inches of rain is expected within six hours.

Two sites, Coyote Creek and San Mateo Creek, have been included because previous trash (debris > 5 mm) research was carried out at these sites in 2015 and 2016 (BASMAA 2016; BASMAA 2014b). Colma Creek is already an RMP site; this site was also monitored for trash during the Tracking California's Trash Project (BASMAA 2014b). A stormwater site managed by the San Francisco Public Utility Commission is also proposed.

Table 7.1 includes a summary of the location and number of stormwater sites. Further details on the exact locations, rationale for site selection and maps of site locations are included in Table B-5.1 in Appendix B-5.

Modeling needs

To better understand the fate and transport of microplastic within the Bay and the exchange with the adjacent Pacific Ocean, it will be important to develop a model of microplastic transport. Key inputs for the modeling effort will be estimates of discharge of microplastics from stormwater and wastewater pathways. This stormwater monitoring effort supplies essential inputs for the particle transport model.

7.4 Field Sample Collection Methods

Stormwater samples will be collected at up to 15 sites in San Francisco Bay, as identified in Table 7.1. Set up for field work will commence prior to rainfall if possible, since in many cases, trash may transport down storm drains and urban rivers relatively quickly. Therefore, sampling as the storm hydrograph builds is particularly important, and field teams will aim to collect the microplastic sample as soon as rainfall begins (and flows increase in waterways).

Each sample will include filtering stormwater from the entire water column collected by an ISCO sampler. The field team will use an ISCO sampler to pump a total of at least 30-50 gallons of stormwater through stacked 125 micron and 355 micron sieves (355 micron sieve will be situated on top of the 125 micron sieve), by collecting 3-gallon to 5-gallon "sips" multiple times throughout a storm, focusing on the rising hydrograph. The sips of water will be pumped over the stacked sieves as they are collected throughout the storm. The number of sips will be a function of the duration of the storm; however, the

goal will be to obtain at least 30 gallons. It may be necessary to increase this volume if insufficient particles are detected in the early sampling attempts.

When site or weather conditions limit the use of the ISCO sampler, an alternate field collection method may be used, such as lowering a 3-gallon stainless steel bucket into the receiving water using a winch to collect surface water samples in the case where the height of the sampling area is too high to use an ISCO pump (e.g., sample collection from a bridge, where it is not possible to safely access the creek or stream). Once collected, the stormwater will be poured through the stacked sieved, as discussed above. This will be conducted multiple times throughout a storm with a priority placed on the rising hydrograph.

A field duplicate will be taken at one site using two sets of sieves; 3-gallon sips will be taken in series with the duplicate sample following the field sample (i.e., 3-gallons sip that is pumped across first sieve set; second 3-gallon sip that is pumped across the second set). A field blank will be collected at one site by placing a set of sieves near the field sample for the duration of the sampling period. When the foil lid is taken off the field sample, the foil lid will also be taken off of the field blank exposed to the air for the same amount of time that the field sample is exposed to air.

Processing of the sieves will occur in the SFEI laboratory post sample event. Microplastics collected in the sieves will be gently washed using distilled water into glass sample bottles prior to shipping to University of Toronto for analyses. Detailed description of the sample collection and processing is presented in Appendix B-5.

7.5 Laboratory Analyses

Microplastic analyses (125 micron and greater)

The stormwater samples will be processed and analyzed for microplastics by the University of Toronto Rochman laboratory. Organic content in the samples will be digested using a 4N KOH solution at room temperature for 14 days. These are methods that have been tested against other extraction options and are cited as the most effective at removing organic material without compromising the integrity of the microplastic (Dehaut et al. 2016; Lusher et al. 2016). After digestion, samples will be filtered through a 10-micron polycarbonate filter and sealed to prevent cross-contamination. Filters will be analyzed via Raman spectroscopy using Particle Finding software that scans each suspect particle across the filter. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, morphology, and chemical composition will be reported. Photographs of the particles will also be included in the data package. Further discussion of methods is presented in Appendix C.

Quality Assurance / Quality Control

In general, laboratory QA/QC will involve best practices (i.e., wearing cotton clothing, filtering in a clean space and not allowing samples to be exposed to the air when possible, using clean glassware and ultrapure water) for reducing procedural contamination, and will use laboratory and field blanks to

account for any procedural contamination. In the laboratory, one laboratory blank sample will be run each day the lab that performs extractions. For batches of more than 10, there will be one lab blank per 10 samples. Lab blank samples will be filtered DI water that has gone through the same procedure for extraction and analyses as the real samples, i.e., digested in KOH, filtered and analyzed via Raman spectroscopy. Microplastic in the field and lab blank samples will be characterized and microplastics of the same type and morphology will be subtracted (blank corrected) from the field samples to account for procedural contamination. The results of the blanks will be reported for all analyses.

Further discussion of methods is presented in the Appendix C.

Stormwater Loadings Estimates

Stormwater flow throughout San Francisco Bay varies during the course of a storm, between storms, and from wet season to wet season. Stormwater samples will be taken throughout an entire storm and will be used to estimate the average concentrations observed during a storm. We will explore whether microplastic loads can be estimated using standard RMP stormwater techniques (Wu et al. 2017) such as the Regional Watershed Spreadsheet Model (RWSM). Using RWSM, we will estimate an average annual volume of water from each of the sampled watersheds. These may be considered first order estimates, as the microplastic concentrations may vary intra- and interannually and we will have a single storm composite to characterize the load. In addition, the RWSM hydrological model has an error range of ±30% for flow. Nonetheless, these first order loads estimates represent a starting point for characterizing loads from watersheds.

Table 7.1 Stormwater Microplastic Samples in San Francisco Bay (Winter 2017 and 2018)			
Location	**Monitoring Sites**	**Priority**	**Collected?** [1]
Central Bay	MMP-Storm-CB-Ash	Priority 1	
Central Bay	MMP-Storm-CB-Line12A	Priority 1	
Central Bay	MMP-Storm-CB-Line12F	Priority 1	Yes
Central Bay	MMP-Storm-CB-Col12H	Priority 2	
Central Bay	MMP-Storm-CB-Col12I	Priority 2	
Central Bay	MMP-Storm-CB-Col12J	Priority 2	Yes
Central Bay	MMP-Storm-CB-Col12K	Priority 1	
Central Bay	MMP-Storm-CB-Col12M	Priority 2	
Central Bay	MMP-Storm-CB-Meek	Priority 1	
Central Bay	MMP-Storm-CB-SFPUC	Priority 1	
South Bay	MMP-Storm-SB-Coyote	Priority 1	
South Bay	MMP-Storm-SB-SM	Priority 1	
South Bay	MMP-Storm-SB-SFC	Priority 1	
South Bay	MMP-Storm-SB-Colma1	Priority 1	Yes
South Bay	MMP-Storm-SB-Colma2	Priority 1	
South Bay	MMP-Storm-SB-Colma3	Priority 1	
South Bay	MMP-Storm-SB-RedCity1	Priority 1	
South Bay	MMP-Storm-SB-RedCity2	Priority 1	
South Bay	MMP-Storm-CB-Alameda	Priority 1	
South Bay	MMP-Storm-SB-Dry	Priority 2	
Lower SB	MMP-Storm-LSB-Matadero	Priority 1	
Lower SB	MMP-Storm-LSB-SanJose1	Priority 2	
Lower SB	MMP-Storm-LSB-SanJose2	Priority 2	
Lower SB	MMP-Storm-LSB-SanJose3	Priority 2	
Lower SB	MMP-Storm-LSB-Guad850	Priority 2	
Lower SB	MMP-Storm-LSB-Guad900	Priority 2	
Lower SB	MMP-Storm-LSB-Guad010	Priority 2	
Lower SB	MMP-Storm-LSB-Guad075	Priority 2	
Lower SB	MMP-Storm-LSB-Guad150	Priority 2	
Lower SB	MMP-Storm-LSB-Guad	Priority 1	Yes
Lower SB	MMP-Storm-LSB-SFC	Priority 1	
Suisun Bay	MMP-Storm-NB-EAntioch	Priority 2	
Suisun Bay	MMP-Storm-NB-LittleBull	Priority 2	
Suisun Bay/Inland	MMP-Storm-NB-MtDiab	Priority 2	
Suisun Bay/Inland	MMP-Storm-NB-Kirker	Priority 2	
San Pablo Bay	MMP-Storm-NB-Refugio	Priority 1	Yes
San Pablo Bay	MMP-Storm-NB-Rodeo	Priority 1	Yes

1 – Depending on the sites selected and weather, the microplastic project will target between 7 and 15 samples

8 Site Maps

Figure 8.1 Microplastic Sampling Sites in San Francisco Bay

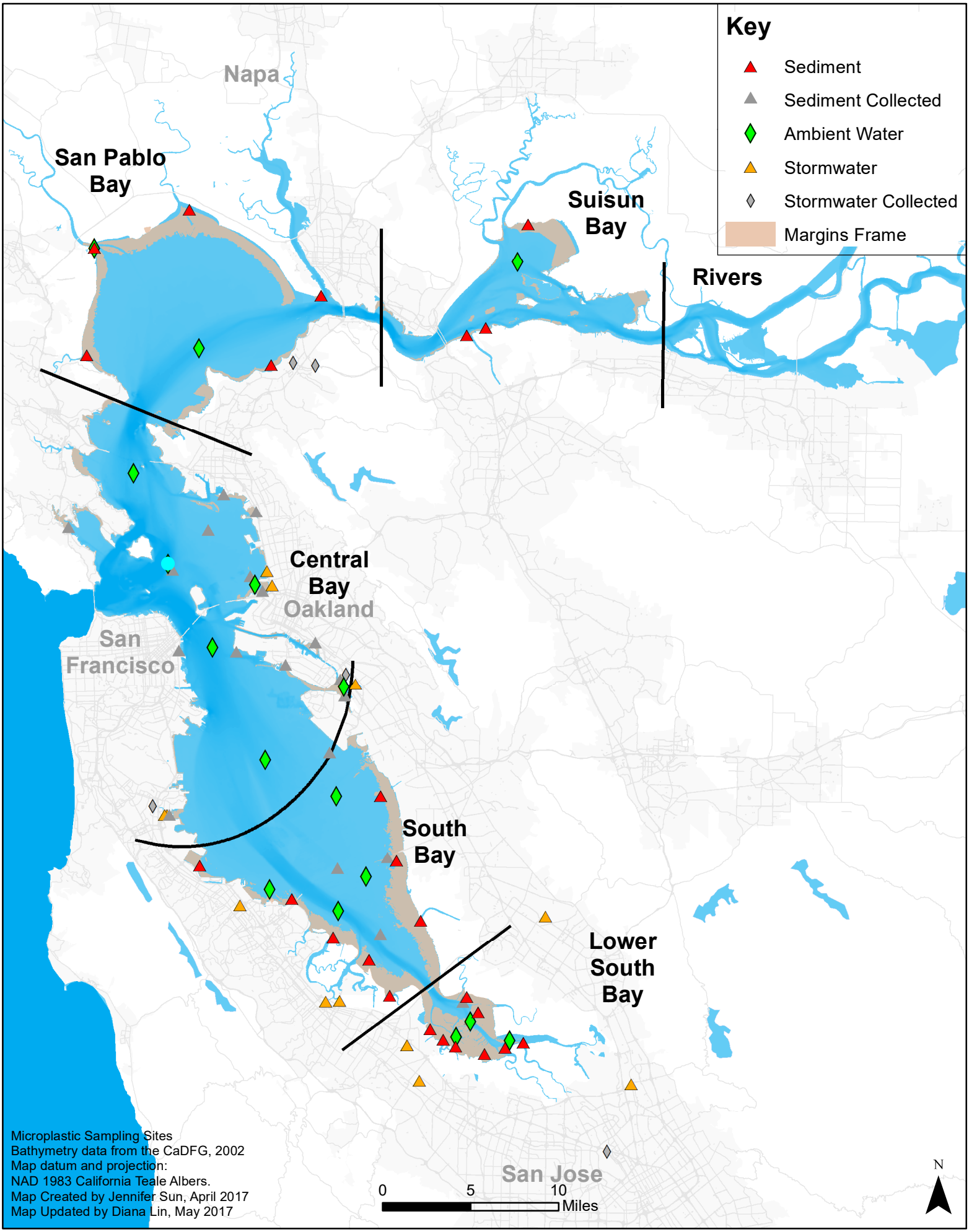


Figure 8.2 Microplastic Sampling Sites in Suisun Bay

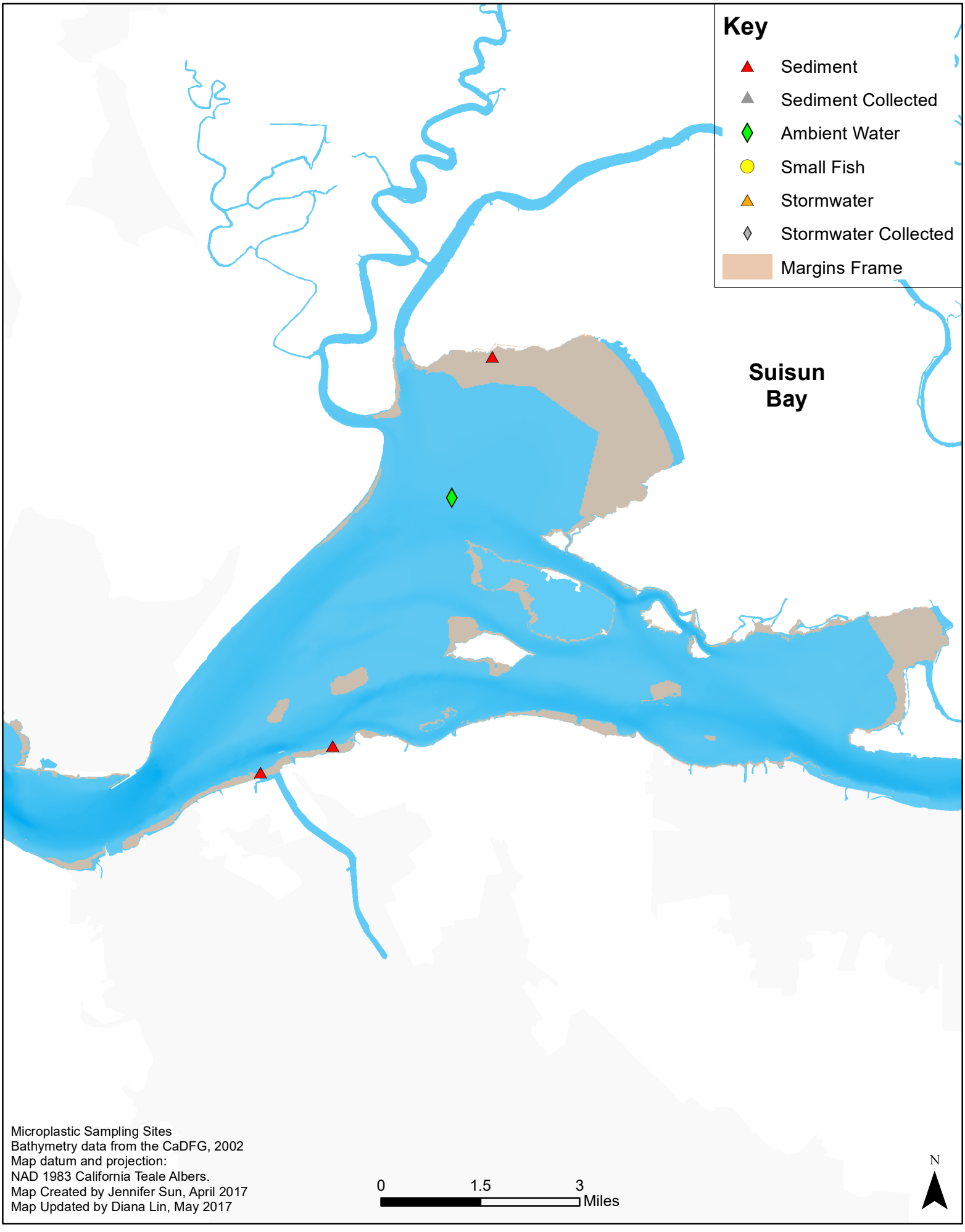


Figure 8.3 Microplastic Sampling Sites in San Pablo Bay

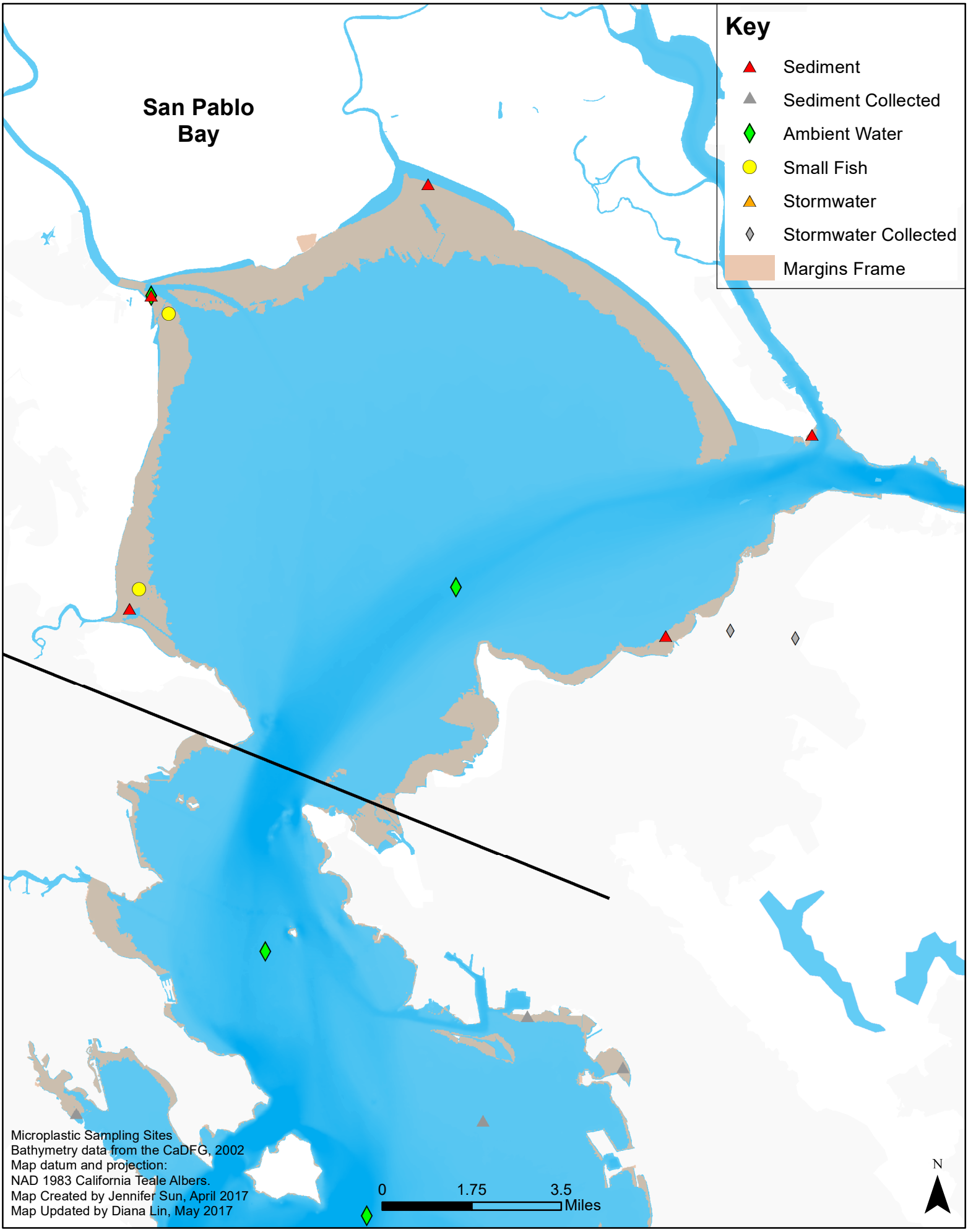


Figure 8.4 Microplastic Sampling Sites in Central Bay

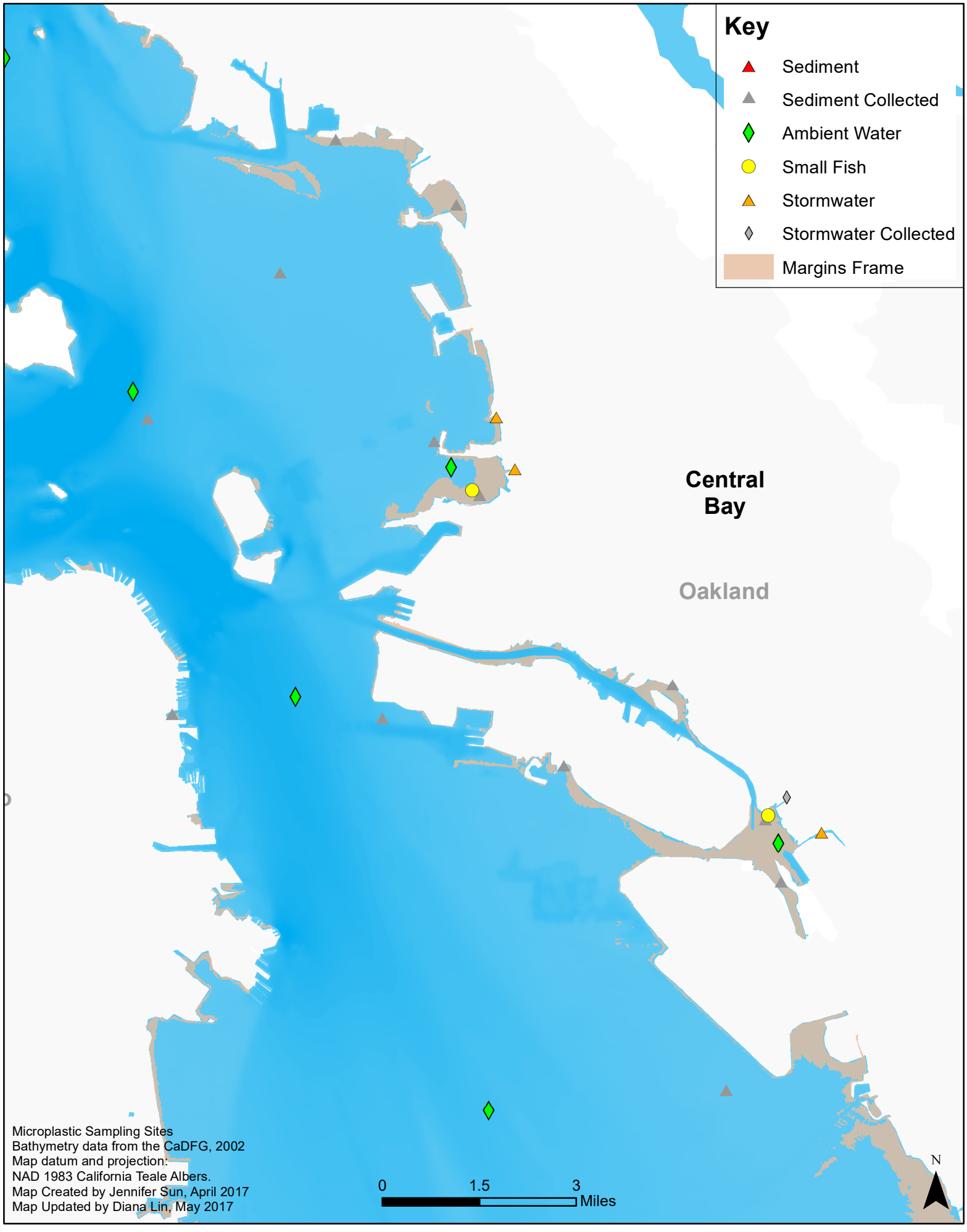


Figure 8.5 Microplastic Sampling Sites in South Bay

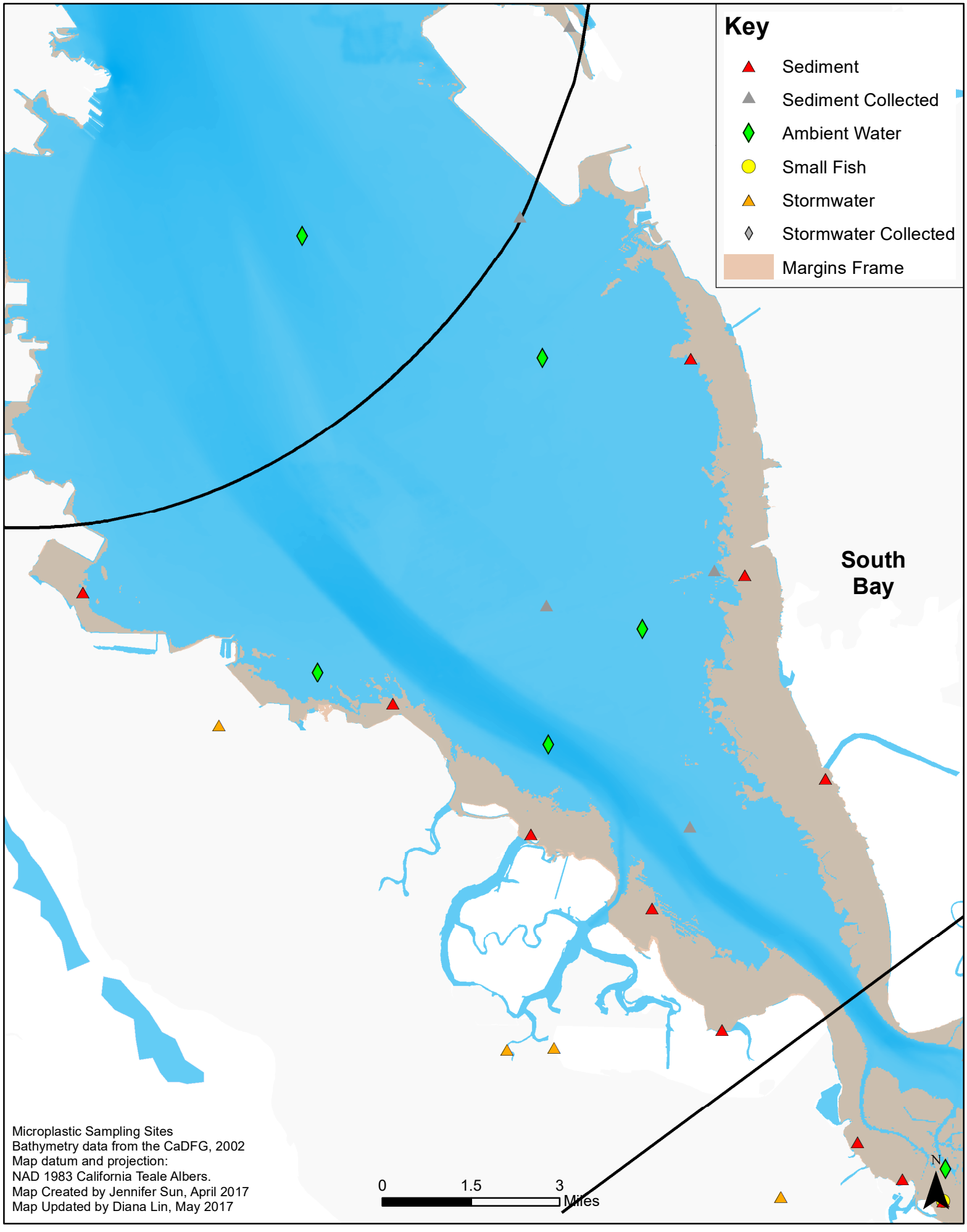


Figure 8.6 Microplastic Sampling Sites in South Bay

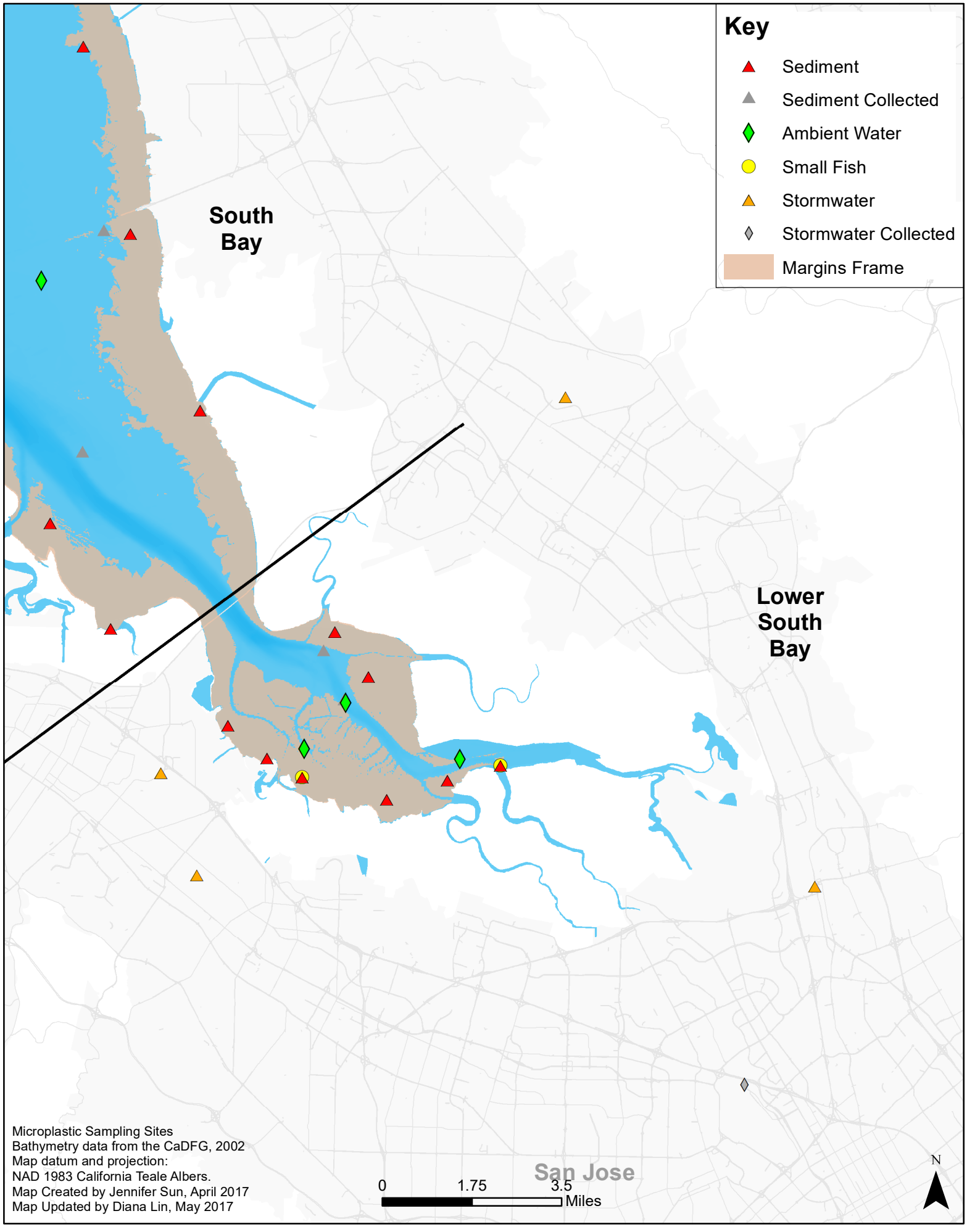
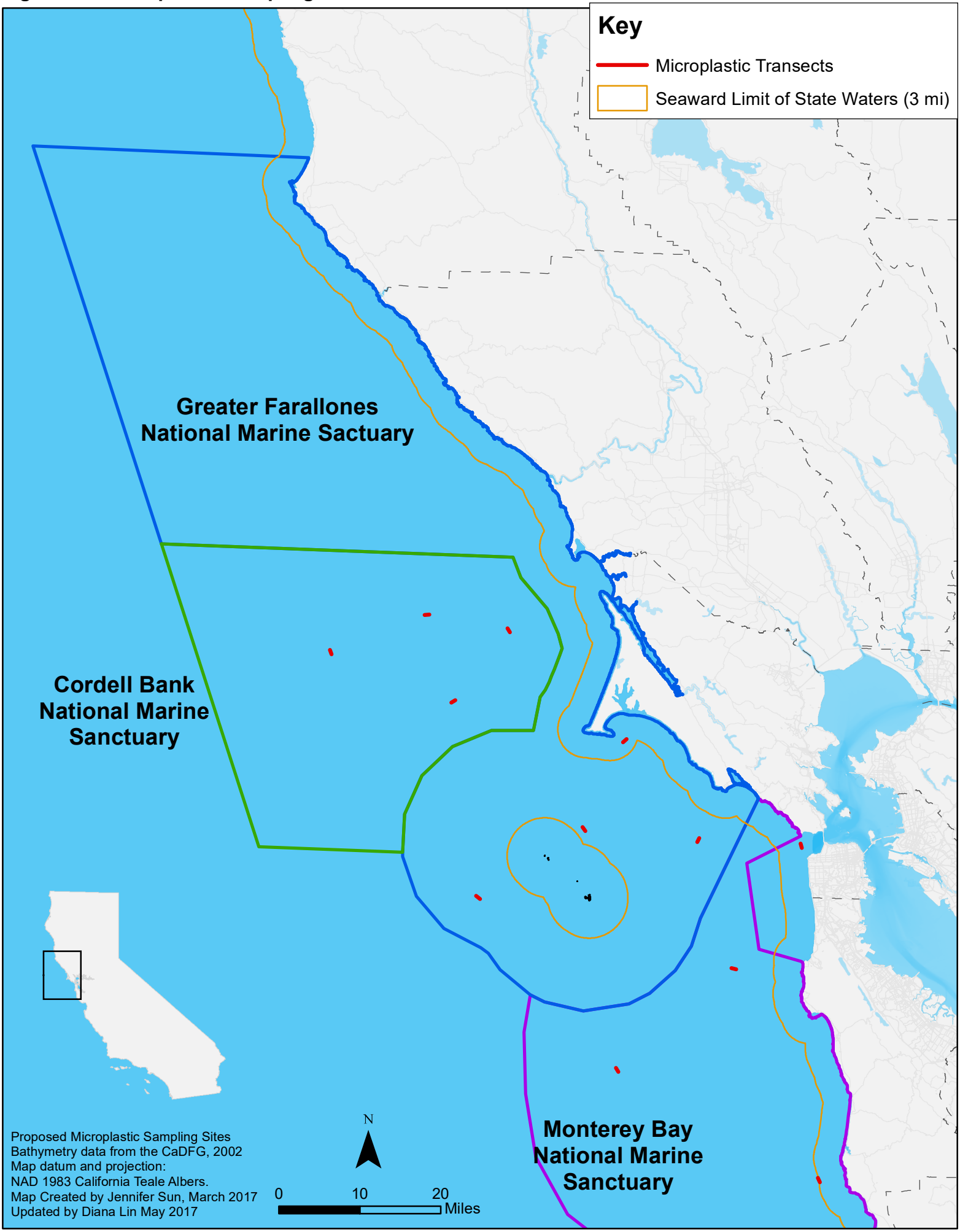


Figure 8.7 Microplastic Sampling Sites in Tomales Bay

Figure 8.8 Microplastic Sampling Sites in the National Marine Sanctuaries



9 Additional Quality Assurance/Quality Control

9.1 Project Organization and Expert Technical Review

The microplastic monitoring project includes multiple partners, as described in Table 9.1.

Project staff will work closely with expert panel members and partners throughout the project, including but not limited to: a) development of the sampling and analysis plan; b) laboratory analysis; c) data processing and analysis; and d) distribution of findings. This will assure that all aspects of the project design and related outcomes are useful, scientifically sound, and aligned with ongoing, related efforts both locally and globally.

9.2 Special Training for the Project

Personnel using field collection equipment will be trained in its use and care, and special instructions related to cross-contamination. Introductory training will be conducted by project managers for all equipment used in the project. All staff involved in field sampling will also undergo safety training prior to working in the field. Sediment, fish, and stormwater data collection will be done in partnership with the RMP; therefore, some training will overlap with previous studies.

Personnel involved in sample collection will have appropriate documentation (e.g., access and collection permits), where needed. The project managers are responsible for providing field equipment operation and safety instruction to all staff in the field. Contractors performing sampling are responsible for providing training to their staff and maintaining records of all trainings. Those records can be obtained if needed from contractors through their respective QA Officers.

Project managers will train field staff, based on the Sampling and Analysis Plan and related project documents. During all aspects of sample collection and sample processing, cross-contamination will be avoided by field and lab staff not wearing textiles that are likely to shed microfibers, keeping containers and samples in clean areas (e.g., covered and in coolers to avoid particles settling out on screens, jars, or samples), and using clean sampling procedures to avoid cross-contamination.

Laboratory analyses will be conducted at University of Toronto and University of Michigan under the supervision of Dr. Chelsea Rochman and Dr. Melissa Duhaime/ Dr. Mark Banaszak Holl, respectively. All analysts will be trained on sample extraction and operation of the analytical instruments (e.g., calibration, maintenance, documentation of sample preparation and analyses).

Table 9.1 Roles and Contact Information

Role	Description of Involvement	Name and Affiliation	Email	Phone Number
Principal Investigator	Head of all research carried out during the project	Rebecca Sutton, SFEI	rebeccas@sfei.org	510-746-7388
Project Manager	Manages all aspects of the project	Meg Sedlak, SFEI	meg@sfei.org	510-746-7311
Data Management and Visualization	Develops and manages samples and all related data	Tony Hale, SFEI	tonyh@sfei.org	510-746-7381
Data Management	Manage data management and distribution	Amy Franz, SFEI	amy@sfei.org	510-746-7394
Baseline Microplastic (Bay and Sanctuary)	Management of baseline research related to microplastics, conduct field work, and development of related documents	Carolynn Box, 5 Gyres	carolynn@5gyres.org	707-328-7942
Baseline Microplastic (Bay and Sanctuary)	Conduct field work and assist in methods development for microplastics	Marcus Eriksen, 5 Gyres	marcus@5gyres.org	323-395-1843
Microplastic Lab Analysis	Analyze microplastic samples related to the project	Chelsea Rochman, University of Toronto	chelsea.rochman@utoronto.ca	647-770-8135
Nanoplastic Lab Analysis	Method development and analyze nanoplastic samples	Melissa Duhaime, University of Michigan	duhaimem@umich.edu	734-764-6219
Baseline Microplastic (Sediment and Fish)	Collection of fish and sediment samples	Rusty Fairey, Moss Landing Marine Lab	fairey@mlml.calstate.edu	831-771-4161
Pathways (Wastewater)	Manage all aspects of wastewater microplastic data collection	Meg Sedlak, SFEI	meg@sfei.org	510-746-7311
Pathways (Stormwater)	Facilitate RMP storm water field work, methods development	Alicia Gilbreath, SFEI	alicia@sfei.org	510-746-7308
Pathways (Stormwater)	Methods development, Field work	Carolynn Box, 5 Gyres	carolynn@5gyres.org	707-328-7942
Transport Modeling	Manage all modeling related to project	Rusty Holleman, SFEI	rustyh@sfei.org	510-746-7387
Transport Modeling	Modeler	Lawrence Sims, SFEI	lawrences@sfei.org	510-746-7333
Policy Recommendations	Facilitate process to evaluate policy options based on project results, generate outreach materials and video	Carolynn Box, 5 Gyres	carolynn@5gyres.org	707-328-7942
Policy Recommendations	Facilitate process to evaluate policy options based on project results, generate outreach materials and video	Anna Cummins, 5 Gyres	anna@5gyres.org	310-998-8616
Policy Recommendations	Write policy briefing based on project results	Chelsea Rochman, University of Toronto	chelsea.rochman@utoronto.ca	647-770-8135
Communications	Manage all aspects of meetings, research publications	Rebecca Sutton, SFEI	rebeccas@sfei.org	510-746-7388

9.3 Health and Safety

Health and safety is the highest priority. Work will be conducted consistent with the San Francisco Estuary Institute Field Work Safety Manual (SFEI 2015) or similar plans developed by contractors for their elements. In addition, work conducted shipboard will follow the health and safety guidance given by the Captain of the vessels. Health and Safety issues will be discussed prior to conducting fieldwork. On vessels, this will be identification of hazards and personal protective equipment (such as life jackets and rafts), and what to do if an incident should occur.

All field staff will be trained prior to entering the field. Field staff will be accompanied by SFEI and 5 Gyres project leads who are well trained and experienced with handling and deploying all equipment included in the project. Field staff will wear boots and gloves when handling equipment.

SFEI's Field Work Safety Manual discusses multiple possible risks such as back injuries and exposure to pathogens that are particularly relevant. Acute back injuries can be the immediate result of improper lifting techniques and/or lifting loads that are too heavy for the back to support. Because pathogens and toxic chemicals in stormwater pose a health risk, puncture and cut-resistant gloves should be worn at all times. Avoid contact with skin, mouth, eyes and nose. After completion of work, immediately wash hands with soap and hot water.

9.4 Permits

All regulatory requirements will be met throughout the project. Regulatory agencies will be contacted and required research permits will be obtained. Table 9.2 describes the research permits that may be needed.

Table 9.2 Potential Permit Requirements

Activity	Regulatory Agencies	Notes
Baseline Microplastic (Bay and Sanctuary)	CA Department of Fish and Wildlife; Sanctuary staff	No permit is necessary for sanctuaries; all three sanctuaries have been notified of project and approved it. CA DFW requests that a scientific collection permit be obtained and any by catch be released promptly. A permit application has been submitted.
Baseline Microplastic (Sediment and Fish)	CA Department of Fish and Wildlife; Sanctuary staff	
Pathways (Stormwater)	CA Department of Fish and Wildlife, local regulartory agencies	RMP permits have been expanded to include microplastics work. Permits will also be obtained by local regulatory agencies for possible work done in Colma Creek, San Mateo Creek and Coyote Creek.
Prey Fish	CA Department of Fish and Wildlife	Permit is required. The subcontractor for this project, Coastal Conservation and Research (CCR), has obtained a Scientific Collection permit.

New permits will be obtained for all components of the project. For stormwater monitoring efforts, the RMP will be adding the microplastic project to existing permits.

9.5 Field Operation and Laboratory Records

The project will collect records for sample collection, field analyses, and laboratory chemical analyses. Appendix B include copies of the field forms that will be used throughout the project to track all data transportation and results. Samples sent to analytical laboratories will include a Chain of Custody form. The analytical laboratories will maintain records of sample receipt and storage, analyses, and reported results and QA/QC reports. The QA/QC reports will include general QC records such as instrument calibration, routine monitoring of instrument capability, verification, etc. Project specific information for QA/QC checks such as blanks and calibration checks will be maintained. SFEI maintains hard copy or scanned files of field notes and measurements, as well as laboratory submitted documentation and results at the SFEI main office. The SFEI Data Manager is responsible for the storage and organization of information. Contract laboratories will also be responsible for maintaining copies of project documentation originating from their respective laboratories, with backup archival storage offsite where possible.

All participants listed in Section 9.1 will receive the most current version of the Project SAP and related documents.

9.6 Data Quality Objectives

Standardized methods for robust microplastic analysis are very much in their infancy, as the scientific community aims to measure smaller and smaller size-fractions and a greater diversity of types of microplastic in a broad range of environmental matrices. One of the goals of this project is to further the work in the field by developing robust scientific methods that can be used for the collection and analyses of microplastic globally. We will be using robust techniques to assure we properly identify microplastic and identify and account for any procedural contamination.

Below we have indicated proposed steps we will undertake to assess the quality of the data produced. As we refine the methods we will use to quantify and identify microplastic in environmental samples during the course of the project, we will update the procedures and QA/QC. As such, the Data Quality Objectives may need to be iteratively refined as we refine methods in each of our matrices of interest.

Goals of the Study

As noted previously, the goal of the study is to characterize (i.e., quantify and type) microplastic in the Bay and National Marine Sanctuaries (e.g., water, sediment, and fish). A second goal of this project is to make inferences about the potential pathways of microplastic to the Bay and Sanctuaries (i.e., wastewater, stormwater). A third goal of this project is to assess the effect of season on detected abundance of microplastic. The Data Quality Objectives are guided by these goals.

Analytical Approach

After extraction, all samples will be filtered onto 10 micron polycarbonate filters, assuring the sample on the filter is homogenous. Each filter will then be transferred to the Raman instrument for analysis. Filters with fewer particles will be analyzed in full. When a filter has too many particles to count, filters will be randomly subsampled.

Spectroscopic analysis proceeds as follows: first, the Raman instrument will take an image of the filter and create a map with each particle on the filter; the size of each particle is recorded. Next, the instrument will analyze each particle using the laser to determine the material type. Raman spectroscopy uses a laser to excite molecules to identify the chemical composition of the particles. Chemical spectra will be generated for each particle and compared to a known library of chemical spectra. It is the goal of the project to pursue spectra matches that are 80 percent or greater; however, there can be interferences such as biofilms that may make this challenging. Photos will be taken of each fragment that is confirmed to be microplastic, and its morphology will be recorded. After analysis, the extracted filter will be archived.

We will use a HORIBA XploRA PLUS Confocal Raman Microscope. The lasers will be aligned and calibrated daily before each use, including testing against a standard reference material.

We are currently evaluating the possibility of finding a second laboratory to conduct verification of the filters containing microplastic using another method such as Fourier Transform Infrared (FTIR) analyses. Secondary confirmation is not common in the field; as such we did not budget this activity. Nonetheless, at the writing of the SAP, we are currently pursuing this possibility.

Quality Assurance / Quality Control Measures

Precision

To evaluate the precision of these analyses, repeat measurements with the laser are obtained for each particle. One of the challenges of using a laser to identify particles is that occasionally the particle will be compromised due to the heat generated by the laser beam.

Recovery

Recovery is a measurement of the ability to obtain a known or previously measured quantity of the contaminant of interest. This is typically measured for chemicals by creating a sample or spiking a sample in a similar matrix with a known amount of a contaminant or analogous compound, or measured in a reference sample previously quantified by one or more external parties. The recovered result is compared to the known or expected amount in the sample. For microplastic, this is a somewhat challenging parameter to assess, as a reasonable standard is difficult to select or create *a priori* because we do not yet know a representative number, type (e.g., fiber, foam, pellet, etc.) and size range (e.g., <5mm, <1 mm, <20 micron) of microplastic we will find in Bay samples. Project staff will evaluate the

typical types, size class, and numbers of microplastics that are detected and then evaluate one or more suitable particle type(s) to be used as a microplastic matrix spike that can assess our recoveries.

Laboratory spiked samples
Once we have identified the typical range of particle sizes, materials, and concentrations seen in environmental samples, we will create a laboratory spiked blank sample that will mimic a "typical" sample from the central portion of the range of results seen. With this lab-created sample, we will evaluate the variability in recovery. Variability in field collected samples will be at least as high and likely higher than in these idealized, relatively simple spiked blank matrix samples, so acceptance criteria for measurement precision will be developed for these samples (e.g., by estimating a 95% confidence interval of mean relative percent difference seen, and flagging deviations above that range).

Blanks and Duplicates
Evaluating blank samples will be crucial for microplastic analyses and will help to quantify procedural contamination that may be introduced as part of sample collection, processing and analyses. Both field and laboratory blanks will be collected to evaluate possible sources of contamination. Quantities in the blank will be subtracted from field samples. Equally important will be an evaluation of the duplicate samples to assess the variation that arises from heterogeneous systems such as sediment and water, field collection, and laboratory analyses. This information will be used to assess variability in the data.

9.7 Treatment of the Data

In the preceding chapters, for each matrix and pathway, we have described the questions we are trying to answer with the proposed sampling plan. This section outlines the methods by which we will analyze the data. This section focuses on microplastic, as the nanoplastic analytical methods are in development. If we are successful in developing robust methods for nanoplastic analyses, it is likely that similar techniques will be applied to the nanoplastic data.

The treatment of data is grouped by similar hypotheses.

Pathways, Fate, and Transport

Hypotheses

- Concentrations of microplastic in the Bay will be higher than in the ocean.
- Within the Bay, concentrations of microplastic will be higher in areas with limited flushing such as the Lower South Bay.
- Concentrations of microplastic in wastewater and stormwater will be comparable; however, the composition of the microplastic will be drastically different (see below Composition).
- Concentrations of microplastic in wastewater effluent will be independent of treatment trains (i.e., secondary vs. tertiary treatment).

- Concentrations of microplastic in stormwater runoff from less developed areas will be less than those from urban areas (e.g., trash hotspots).
- Concentrations of microplastic in sediment will be less in remote areas such as Tomales Bay in comparison to sediment from the San Francisco Bay.
- Concentrations of microplastic in sediment from the main channel of the Bay will be less than from the Bay margins.

If Bay surface water is found to have higher concentrations of microplastic than the Sanctuaries, this would suggest that the Bay is a net source of microplastic to the ocean. Similarly, if concentrations of microplastic in Bay sediments are higher than concentrations of microplastic in remote coastal areas (Tomales Bay), this would suggest again that the Bay is a source of microplastic. If the main channel of the Bay has lower concentrations of microplastic, this would suggest that the margins accumulate microplastic

Metric

Microplastic will be enumerated into the following size categories:

- Above 5 mm
- 5 mm to 355 micron
- 355 to 125 micron
- 125 to 20 micron

It is possible that after the data is reviewed, additional refinements to the size categories will be warranted.

Data Analysis Techniques

The data will be presented graphically using box-whiskers plot of concentrations for each size category for wastewater effluent, stormwater, Bay, and sanctuary samples. Direct comparisons between pathway water samples and ambient Bay and Sanctuary water samples may not be appropriate because we are monitoring the surface water of the Bay and Sanctuary, whereas the effluent samples represent a snapshot of the entire discharge. Similarly, for stormwater, we will attempt to capture a distribution of microplastic in the whole water column by moving the collection tube through the water column during sampling.

If the data are normally distributed, we will use student t tests or analysis of variance. If the data are not normal, non-parametric statistical methods such as Wilcoxon or Kruskall Wallis test will be applied with significance level of $p<0.05$.

DQO/Acceptable Error

The data quality objective for this element is to be able to detect a 50% difference in the central tendency concentration between source type (e.g., effluent vs stormwater), water type (Bay vs. ocean),

spatial distribution as a proxy for flushing (e.g., Lower South Bay vs North Bay) for at least one size-type category of microplastic (e.g., fibers, fragments, etc.).

Differences in particle counts between water sites near pathways such as urban creeks and effluent discharges and other sites in the Bay will be difficult to detect due to the small number of sites included in this study; however, we may be able to make qualitative inference as a result of the morphology (e.g., fragment vs fiber), chemical composition (e.g., polyester vs polystyrene), etc.

Composition

Hypothesis

- Different pathways will deliver different types of microplastic.
- Water and sediment from the same location will contain different types of microplastic (e.g., morphology and chemical composition).
- Margin sediments will exhibit a different type of microplastic than the main channel (e.g., morphology and chemical composition).
- Sediments near creeks and wastewater outfalls will show a different pattern than background Bay or Tomales sites

If these hypotheses are confirmed, then management of sources to pathways will likely need to be different for different types of sources. For example, we expect to see fibers from wastewater treatment facilities; polyester is a common type of microfiber observed in wastewater treatment effluent. If true, management actions are likely to focus on means for reducing the shedding of fibers or abrasion of textiles. Conversely, urban creeks are more likely to have fragments from large pieces of plastic (e.g., polystyrene foam) which is not frequently detected in effluent. If this hypothesis is confirmed, then management actions may target specific types of plastic (e.g., polystyrene foam) or better management of trash.

Metric

Descriptive variables for this metric include:

- size fraction (e.g., count and percent of particle in a given size fraction);
- morphology (e.g., fiber, fragment, pellet, etc.) specified by count and percentage;
- chemical composition (e.g., polystyrene, polypropylene, polyester, etc.)

Data Analysis Techniques

The data will be evaluated using principal component analyses to narrow or aggregate the number of significant parameters. Cluster analyses may also be employed. Depending on the outcome, statistical tests will be used to determine the significance.

Seasons

Hypothesis

- Concentrations of microplastic will be higher in the wet season.

If confirmed, this difference suggests that wet weather events increase microplastic concentrations through increased transport of microplastic from pathways, the possible fragmentation of large-sized trash to microplastic during high energy storm events, and the potential washoff of airborne microplastic from road surfaces, agricultural fields that may have sources of plastic (e.g., deposition of biosolids, degraded plastic sheeting, etc.), and airborne microplastic from other sources.

Metric

Microplastic will be enumerated into the following size categories:

- Above 5 mm
- 5 mm to 355 micron
- 355 to 125 micron
- 125 to 20 micron

Data Analysis Techniques

The data will be presented graphically using box-whiskers plot of concentrations for each size-type category for wet versus dry seasons for the water samples collected from the Bay and Sanctuaries. The data will be tested for differences in the metric between wet and dry. Non-parametric statistical methods such as Wilcoxon or Kruskall Wallis test will be applied with significance level of $p<0.05$.

DQO/Acceptable Error

The data quality objective for this element is to be able to detect a 50% difference in the central tendency concentration between Bay samples and the ocean samples for at least one size-type category of microplastic.

Bioaccumulation

Hypothesis

- Microplastic will be present in fish.
- Fish from reference sites will have lower concentrations of microplastic.
- Sediment and fish from the same location will have comparable distributions of microplastic (e.g., morphology and chemical composition).
- Fish habitat will affect microplastic uptake. Pelagic species (e.g., anchovy) will have a different microplastic composition than fish that reside largely in the margins (e.g., topsmelt and Mississippi silverside).

Metric

Descriptive variables for this analysis include:

- size fraction (e.g., count and percent of particle in a given size fraction);
- morphology (e.g., fiber, fragment, pellet, etc.) specified by count and percentage;
- chemical composition (e.g., polystyrene, polypropylene, polyester, etc.)

Data Analysis Techniques

The data will be evaluated using linear regressions (e.g., particle count vs microplastic average by site). Depending on the distribution of the data (normality), we will consider analysis of variance for the comparison among different species. Analyses may involve developing a statistical model that considers the random effect of site and the fixed effect of fish species, sediment count, fish count, etc.

DQO/Acceptable Error

This is not known at this time.

9.8 Data Reporting

This project will collect environmental data consisting of basic field data (e.g., time, date, field staff, and basic field parameters) and laboratory microplastic analyses (e.g., counts of microplastic per sample) including Raman spectra and photographic records of particles collected and identified as plastic.

The field data will be collected by staff on field data sheets. Field sheets and the sampling and analyses plan will be used to prepare an electronic data deliverable (EDD) template supplied by SFEI to the analytical laboratory. The data template will be populated with analytical and QA/QC results by the laboratory and then submitted electronically to SFEI to undergo data validation (e.g., checking expected number of samples, data type, station names/location, review of available quality assurance and quality control (QA/QC) data).

The data will be converted to a standard CEDEN/SWAMP database format, entered into the San Francisco Bay Regional Data Center (RDC) database, hosted by SFEI, and in turn uploaded to the California Environmental Data Exchange Network (CEDEN) (ceden.org) and displayed on SFEI's Contaminant Data and Download Display (CD3) site (cd3.sfei.org). This will be breaking new ground as the CEDEN template for microplastic is quite limited and will need to be modified. This aspect of the project may be quite challenging as we develop templates and methods for uploading this new type of data.

Tabulated data will include the following information for each sample (when applicable):

1. Sample identification: Unique sample ID, site code, site name, collection date, analysis date, sample type (field or QA/QC types), and matrix (water, sediment, tissue (include species code)).
2. Analytical methods: Preparation, extraction, and quantitation methods. Also include preparation, extraction, and analysis dates.

3. Analytical results: Analyte name, result, unit, method detection limit (MDL), and reporting limit for all target parameters. The appropriate data qualifiers should be submitted with the results.
4. QA/QC results, including field and method blank sample results.

SFEI is a Regional Data Center (RDC) for the state of California and uses templates, standardized vocabulary and business rules developed and maintained by the California Environmental Data Exchange Network (CEDEN) to manage data for field collection, chemistry, taxonomy, tissue, toxicity, and bioassessment sampling. SFEI will work with the CEDEN community to develop standardized vocabulary specific to microplastic characterization, as this contaminant is not currently represented in the CEDEN database. Vocabulary must address relevant categories of microplastic in terms of size range, particle shape or type, and polymer type.

The environmental data will be used as input data for an associated modeling exercise. Model graphical output will be in georeferenced KML format, with tabulated summary statistics in Excel spreadsheets. An additional, technical archive of the modeling results will be generated as NetCDF. These files will be available through an archive on http://www.sfei.org/data-center.

As detailed above, data generated by this project will be made publicly available in accordance with the Data Sharing Plan established with the Gordon and Betty Moore Foundation.

9.9 Storage/Archiving

Sediment samples will be collected for an archive in the event that additional material is needed for analyses; this material will be stored at Moss Landing Marine Labs. Fish caught in nets in excess of the targeted amounts will also be stored at Moss Landing Marine Labs until completion of the project. Microplastic extracted on to filters will be retained until completion of the project at the analytical laboratory. Where possible, the portion of fish tissue (e.g., muscle tissue) and sediment (excess sediment) that is not extracted for the project will be retained.

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Appendix A

Gordon and Betty Moore Foundation Outcome Table

ATTACHMENT 1
GRANT OUTCOMES, PAYMENTS AND REQUIREMENTS (#5598)

OUTCOME TABLE				
Due Date	***Act/Ind***	***Description***	***% Complete***	***Progress Report***
5/2019	***OUTCOME 1: New knowledge and scientifically accurate data that quantifies and characterizes the dimensions of microplastic pollution in San Francisco Bay and nearby areas of the Pacific Ocean, and is provided to industry leaders, scientists, conservationists, and policymakers***			
11/2018	***Output 1.1: BASELINE DATA – Quantified dimensions and parameters of microplastic pollution in surface water (including adjacent ocean areas), Bay sediment, and fish using scientifically replicable techniques, including existing and new protocols***			
5/2018	***1.1.1***	Surface water – Characterize baseline microplastic contamination in surface water samples in San Francisco Bay and adjacent ocean environment including Marine Sanctuaries (e.g., Greater Farallones, Cordell Bank, Monterey Bay) during dry and wet season, using existing protocols. Evaluate new pump system to characterize microplastics down to 20 micron range. Collect samples in ocean and Bay to be analyzed for nano-microplastics using novel techniques.		
5/2018	***1.1.2***	Sediment – Characterize baseline macro- and microplastic contamination in sediment samples from the ambient Bay as well as from the Bay margins (near-shore environments), using existing protocols.		
5/2018	***1.1.3***	Fish – Characterize microplastic in benthic and pelagic prey fish, considering new EPA protocols to measure microplastic pollution in fish.		
3/2017	***Ind.***	• Sampling plans for surface water, sediment and fish		
1/2018	***Ind.***	• Collection of surface water samples – Wet/Spring 2017 or Wet/Winter 2017; Dry/Summer 2017; Sediment sampling – Summer 2017; Fish – Summer and Fall 2017		
1/2018	***Ind.***	• Laboratory and data analyses		
5/2018	***Ind.***	• Year 1 baseline report, including baseline data on microplastics in fish, surface water, and sediment in the Bay and surrounding ocean waters, and contributions to standardized methodologies and new tools		
11/2018	***Output 1.2: PATHWAYS - Improved characterization of pathways by which microplastic enters San Francisco Bay***			

ATTACHMENT 1
GRANT OUTCOMES, PAYMENTS AND REQUIREMENTS (#5598)

OUTCOME TABLE				
Due Date	***Act/Ind***	***Description***	***% Complete***	***Progress Report***
10/2018	***1.2.1***	Characterize the quantity, distribution, and types of microplastic contaminants in treated wastewater entering SF Bay and surrounding ocean areas using improved methods (NOAA method as modified by BACWA, including spectroscopic polymer identification).		
10/2018	***1.2.2***	Characterize the quantity, distribution, and types of microplastic contaminants in stormwater by monitoring mouth of tributaries after major storm events or if suitable methods have been developed, sample directly in watersheds.		
12/2017	***Ind.***	• Sampling plan for wastewater effluent and stormwater		
5/2018	***Ind.***	• Collection of effluent and storm water samples – Wet/Winter 2017; Dry/Spring 2018		
8/2018	***Ind.***	• Laboratory and data analyses		
12/2018	***Ind.***	• Year 2 Baseline report		
11/2018	***Output 1.3: TRANSPORT MODEL - A transport model to predict microplastic contamination levels in the Sanctuaries based on Bay regional inputs***			
4/2018	***1.3.1***	Modify existing Bay hydrodynamic model currently being used for nutrients and water quality issues in the Bay (Open source Delft 3D model). Couple this model with existing particle transport models outside of the Golden Gate. Incorporate realistic assumptions for microplastic loss from surface water due to settling, ingestion by biota, and other fates.		
11/2018	***1.3.2***	Generate spatial and temporal maps of predicted microplastic concentrations, and evaluate model predictions using baseline data from adjacent ocean sites.		
11/2017	***Ind.***	• Modifications of existing Bay model to incorporate particle tracking		
1/2018	***Ind.***	• Bay model coupled to particle transport models outside Golden Gate		
4/2018	***Ind.***	• Reviewed model assumptions with expert panel		

ATTACHMENT 1
GRANT OUTCOMES, PAYMENTS AND REQUIREMENTS (#5598)

OUTCOME TABLE				
Due Date	***Act/Ind***	***Description***	***% Complete***	***Progress Report***
11/2018	***Ind.***	• Spatial and temporal maps. Evaluation relative to baseline data		
1/2019	***Output 1.4: POLICY AND INNOVATION OPTIONS - Public and private sector policy and innovation options to reduce microplastic pollution***			
11/2018	***1.4.1***	Evaluate public policy options and private sector innovations that may reduce microplastic entering SF Bay (either quantitatively or qualitatively), in light of improved data, and incorporate into 5 Gyres community campaigns.		
11/2018	***Ind.***	• Convening with group of experts to evaluate current policy options for SF Bay (local bans, CA trash policy, etc.)		
12/2018	***Ind.***	• Policy synthesis and innovative options that could be applied in SF Bay		
1/2019	***Ind.***	• Educational materials that can be used in community campaigns		
5/2019	***Output 1.5: COMMUNICATIONS - Findings disseminated to key audiences***			
12/2017	***1.5.1***	Document research expeditions (Year 1) via video capture and post targeted materials on SFEI and 5 Gyres websites. Host selected industry and government leaders aboard surface water expeditions (Year 1).		
2/2019	***1.5.2***	Document research findings from 1.1-1.3 in scientific manuscript(s) for peer-reviewed journal. Provide funding to ensure "Open Access" to manuscript (at no cost to the public). Incorporate up-to-date science on microplastics, and scientifically-based information on the impact to SF Bay and surrounding ocean areas, and comparison to other places, where appropriate.		
1/2019	***1.5.3***	Synthesize findings to allow broader applications, (e.g., lessons learned, best practices for monitoring, potential impacts to SF Bay and ocean, etc.) and publish in appropriate outlets.		
1/2019	***1.5.4***	Recommend additional research priorities to further improve scientific understanding of sources and pathways of microplastic pollution in the marine environment, broadly and for the region.		

ATTACHMENT 1
GRANT OUTCOMES, PAYMENTS AND REQUIREMENTS (#5598)

OUTCOME TABLE				
Due Date	***Act/Ind***	***Description***	***% Complete***	***Progress Report***
1/2019	***1.5.5***	Organize a one-day symposium for private, public, and NGO sector leaders following Year 2 work to discuss findings, new tools, and policy/innovation implications. Conduct follow-up survey quantifying awareness and understanding of issues surrounding microplastic.		
5/2019	***1.5.6***	Disseminate findings to private, public, and NGO leaders via targeted presentations with tailored messages at conferences or meetings of major stakeholders.		
12/2017	***Ind.***	• Video of cruises, posted on websites		
4/2019	***Ind.***	• Submitted manuscripts		
12/2018	***Ind.***	• Outreach materials summarizing findings to public, media, and decision makers		
1/2019	***Ind.***	• Upload microplastic data to CEDEN for public download and display		
1/2019	***Ind.***	• One-day symposium at external venue		
11/2019	***Ind.***	• Grantee participation in RMP (Regional Monitoring Program) Annual Meeting – Fall 2019. Attendance at national conferences		
5/2019	***Ind.***	• Surveys assessing impacts of findings on key audiences, including scientists, managers, conservation organizations, industry, and decisionmakers		

ATTACHMENT 1
GRANT OUTCOMES, PAYMENTS AND REQUIREMENTS (#5598)

GRANT PAYMENTS & REQUIREMENTS						
GRANT PAYMENTS			***GRANT REQUIREMENTS***			
(A) Scheduled Payment Date	***(B) Payment Number*** ⋆	***(C) Amount***	***(D) Requirement Due Date***	***(E) Type of Requirement***	***(F) Description of Report / Activity***	***(G) Contingency for release of Payment #***
11/15/2016	Payment #1	$880,250	11/8/2016	Signed Contract	Fully signed and executed Grant Agreement	Payment #1
			2/1/2017	Meeting	A kick-off meeting with Foundation staff to share updates on grant activities and progress towards the outcomes.	
			7/1/2017	Project Documentation	Brief (1–2 page) project update describing progress towards achieving the Grant objectives and project challenges through 6/1/2017.	
			7/1/2017	Cash Position Statement	Cash balance report reflecting expenditures to date, grant funds remaining, and accrued interest through 6/1/2017.	
			7/1/2017	Meeting	Meeting with Foundation staff to share updates on grant activities and progress towards the outcomes, including discussion of the project update and cash position statement.	
			12/1/2017	Annual Narrative and Financial Grant Reports	Submission of Annual Narrative and Financial Grant Reports for Year 1 (grant start to 11/1/2017). The Foundation will provide a report template at least 1 month in advance of its due date. The narrative will include an update on grant activities and progress towards the outcomes. Beyond a narrative, it will include the Attachment 1 Outcome Table with % Complete and Progress Report columns filled out. It will also include a comparison of budget to actual expenditures from grant inception through 11/1/2017, at the level of expense category in the budget (Attachment 2), a description of variances greater than 20%, and an update on Other funding raised to support the project.	
			5/1/2018	Data Sharing Update	Public release of laboratory protocols, experimental data, and code generated during Year 1 (grant start date to 11/1/2017), per provisions in data sharing plan.	
			5/1/2018	Meeting	Phone or in-person midpoint meeting with Foundation staff to share updates on grant activities and progress towards the outcomes, including challenges.	

⋆ *See* Column (G) for the requirements that are contingencies for release of payments.

Appendix B
Field Procedures

Appendix B-1
Field Procedures for Surface Water Collection
San Francisco Bay and National Marine Sanctuaries

1. Sampling Design (See Water Cruise Plan for latest information)

Table B-1.1. Rationale and location of Surface Water Microplastic and Nanoplastic Samples in San Francisco Bay (Summer / Winter 2017)

Monitoring Site	Location	Subregion	Target Latitude	Target Longitude	Rationale for site selection	Description
MMP - LSB - GR	Lower South Bay	Near Guadalupe River	37.4637	-122.03625	Receiving water for tributaries; wastewater	Samples should be taken just after high tide as tide drops (ebb tide)
MMP - LSB - PA	Lower South Bay	Near Palo Alto WWTP	37.46568	-122.09164	Receiving water near wastewater	
MMP - LSB - MAIN	Lower South Bay	Main stem of LSB	37.47889	-122.07722	Ambient conditions in LSB embayment	
MMP - SB - MAIN - SE	South Bay	Main portion of Bay - Southeast	37.59613	-122.18876	Ambient conditions in SB embayment	Sample east of deep channel
MMP - SB - SM	South Bay	Near San Mateo creek	37.58367	-122.28866	Receiving water for tributaries	On shoal - west of deep channel
MMP - SB - MAIN - NE	South Bay	Main portion of South Bay - Northeast	37.6619	-122.2216	Ambient conditions in SB embayment	Sample in east of deep channel
MMP - SB - MAIN - SW	South Bay	Main portion of South Bay - Southwest	37.5673	-122.217	Ambient conditions in SB embayment	Sample in deep channel
MMP - CB - OUTFALL	Central Bay	Main portion of Bay - Near EBDA outfall	37.69041	-122.29661	Receiving water for WWTP-EBDA	Start at outfall and head towards East Bay
MMP - CB - SL	Central Bay	San Leandro Creek / Oakland Airport	37.75187	-122.21649	Receiving waters for tributaries	Collection starts in San Leandro Bay and towards Oakland Airport
MMP - CB - EMERY	Central Bay	Emeryville	37.83439	-122.31173	Receiving waters for tributaries	Sample adjacent to the Bay Bridge toll booths
MMP - CB - BAYBRIDGE	Central Bay	South of Bay bridge	37.78198	-122.35419	Ambient conditions	Middle of Bay
MMP - CB - ANGEL	Central Bay	Main Channel in Central Bay, Southeast of Angel Island	37.8496	-122.40235	Ambient conditions	Centered between Angel Island and Treasure Island
MMP - CB - RICH	Central Bay	South of Richmond / San Rafael bridge	37.92362	-122.44111	Ambient conditions	Middle of Bay
MMP - NB - PR	North Bay	Petaluma River	38.08691	-122.25274	Receiving water for tributaries	Collected during ebb tide
MMP - NB - SUISUN	North Bay	Suisun Bay main	38.10521	-122.0455	Ambient conditions	Sample in Grizzly Bay
MMP - NB - SANPABLO	North Bay	San Pablo Bay main	38.02811	-122.37601	Ambient conditions	Sample in channel

Table B-1.2. Rationale and location of Surface Water Microplastic and Nanoplastic Samples in National Marine Sanctuaries adjacent to San Francisco Bay (Summer / Winter 2017)							
Monitoring Site	**Location**	**Subregion**	**Start Point - Target Latitude**	**Start Point - Target Longitude**	**End Point - Target Latitude**	**End Point - Target Longitude**	**Rationale for site selection**
MMP - CORD - S	Cordell Banks	South side	38.03727	-123.32001	38.04113	-123.32001	Ambient conditions
MMP - CORD - N	Cordell Banks	North side	38.19126	-123.38567	38.19277	-123.38567	Sample drift algae
MMP - CORD - E	Cordell Banks	East side	38.17303	-123.20163	38.16613	-123.20163	Ambient conditions
MMP - CORD - W	Cordell Banks	West side	38.12281	-123.60439	38.11508	-123.60439	Ambient conditions
MMP - FAR - C	Greater Farallones	Farallon Islands	37.82250	-123.01671	37.81562	-123.01671	Ambient
MMP - FAR - GG	Greater Farallones	At discharge of GG; SF Plume	37.80948	-122.76256	37.80180	-122.76256	Modeling; Load Calculations
MMP - FAR - S	Greater Farallones	Off of Point Reyes	37.97593	-122.92985	37.98190	-122.92985	Convergence zone off of Pt Reyes
MMP - FAR - W	Greater Farallones	West side	37.69320	-123.24921	37.68809	-123.24921	Remote part of Greater Farallons - reference comparison
MMP - MONT - GG	Monterey Bay	At discharge of GG; SF Plume	37.80456	-122.52680	37.79657	-122.52680	Modeling; load calculations; outgoing tide
MMP - MONT - OB	Monterey Bay	At discharge of GG; SF Plume	37.57846	-122.66740	37.57655	-122.66740	Modeling; load calculations
MMP - MONT - S	Monterey Bay	Off the coast of Ano Nuevo	37.20863	-122.46663	37.20110	-122.46663	Upwelling areas around Pt Ano Nuevo
MMP - MONT - W	Monterey Bay	West site	37.39477	-122.92508	37.38761	-122.92508	Remote part of Monterey Bay - reference for comparison

Table B-1.3 Field blanks and duplicates				
Matrix	**Collection Method**	**Field Blanks**	**Field Duplicates**	**Comments**
Bay water	Manta	4	4	2 field blanks in Bay (16 sites) for each season (wet and dry). Two duplicate Manta trawls will be taken serially (1 Bay – wet and dry).
	Pump	2	2	
	Nano	2	2	
Sanctuary	Manta	2	2	1 blank will be taken in the Sanctuaries (12 sites) per season (wet and dry). Duplicate Manta trawl will be taken serially (1 sanctuary).
	Pump	2	2	
	Nano	2	2	
Sediment	Grab	4	3	Field blank - DI water will be poured over sampling equipment into pre-cleaned bottle. At 3 sites, fill a second bottle for field duplicate; no archive samples at duplicate sites
	Nano	4	3	
Fish	Net	None	None	
Stormwater	Grab across hydrograph	1	1	Field blank – field blank sieves will remain uncovered as the stormwater sample is being collected. Duplicate - serial sips across the storm, alternating between two different sieve sets.
Wastewater	24-hr composite	1	1	Field blank - sieve uncovered for 24-hour composite period. Field duplicate will be collect using Y splitter off of sampling port to two different sieve sets.

2. Cruise schedule

The cruise schedule is shown in Table B-1.4. The schedule is for planning purposes only, and may be revised during sampling operations to reflect weather conditions, tide restrictions, equipment performance, or other factors. (At the writing of this SAP, we are working with Captains and organizations to see which vessels can best meet our needs for microplastic collection.) Any sites that cannot be sampled at the scheduled time will be sampled later in the cruise, if possible.

Table B-1.4 Anticipated Cruise Schedule for 2017 Microplastic Sampling in Sanctuaries and Bay

Date	Time	Vessel	Activity
Summer 2017 and Fall 2017	TBD	RV Snavely	San Francisco Bay. Tentatively scheduled to collect dry weather samples in all four embayments during Summer (likely June or August) 2017, and carry out wet weather sampling in Fall 2017.
September 2017	TBD	RV Derek M Baylis	Possible option for some Monterey Bay sites.
June / July 2017 and Fall 2017	TBD	RV Derek M Baylis	National Marine Sanctuary sites. Needs to be confirmed.

3. Detailed Field Sampling Methods

Baseline microplastic and nanoplastic pollution monitoring in San Francisco Bay and adjacent National Marine Sanctuaries will be performed during periods of dry weather and after wet weather events. Sample locations for San Francisco Bay sites described in the Table 3.1 and Figure 8.1, and sanctuary sites are described in Table 3.2 and Figure 8.8. Rationale and additional details about monitoring sites are described in Tables B-1.1 and B-1.2.

Generally, Manta trawl and nanoplastic samples will be collected at each sample location, and pump system samples will be collected at a subset of the sites. The following steps should be conducted at each site:

Field Parameters:

1. For latitude and longitude, use the most accurate reading available on the vessel and include all available decimal points.

2. Temperature and salinity should be taken using a Water Quality Data Sondes for each sample and recorded on the *MMP Project Monitoring Event Field Form*.
3. Wind speed and direction should be taken using an Anemometer or by a similar piece of equipment.
4. Boat speed and heading should be recorded.
5. Sea State should be based on the Beaufort Scale of Sea State. Include direction of the dominant waves and approximate wave height.
6. Water speed and direction should be recorded using the vessel's instrumentation.
7. Last rainfall date and time should be recorded.
8. Distance traveled can be recorded by vessel GPS equipment or a knotmeter.
9. Any deviations from Sampling Analysis Plan should be documented in the notes section of the MMP *Project Monitoring Event Field Form.*

Microplastic Sampling with Manta Trawl (355 micron and above):

1. Manta trawl sampling will occur at each monitoring site.
2. All details listed on the *MMP Project Monitoring Event Field Form* will be completed and recorded for each sample collected during the project. For each sample, record Sample ID, Site ID, latitude and longitude at the beginning and end of sample collection, wind speed / direction, sample collection date and time, equipment type, water temperature, salinity, water current speed and direction, last rainfall, sea state, approximate wave height and direction, salinity, field staff, and weather condition, at a minimum. Consistent Sample IDs will be recorded and clearly identified on each sample. Photographs will be taken of each sample and video will be recorded during each sampling event.
3. Manta Trawl Assembly – Assemble the Manta trawl and attach the cod end. Make sure all fasteners, such as hose clamps and wing nuts, are tight. Use two hose clamps to hold the net to the plastic pipe and two clamps to hold the pipe to the cod end.
4. Attach General Oceanics flowmeter across the mouth of the Manta Trawl.
5. Record appropriate data on the *MMP Project Monitoring Event Field Form*). At a minimum, this information should include: Sample ID, Site ID, Date, Latitude and Longitude, Flowmeter beginning time, Wind Speed / Direction, Equipment Type, Time, Water Temperature, Current information, Last Rainfall, Sea State, Wave information, Salinity, and Field Staff.
6. Deployment – Record Start Time, Latitude and Longitude, Boat Speed Beginning and the number on the flow meter. Deploy trawl out of the wake zone. Turbulence inside the wake zone does not allow for a representative surface sample to be collected. Watch the net to observe its performance and adjust to sample the surface properly.
7. Trawl Speed/Direction – Maintain a steady linear course at 1 to 2 knots (max 3 knots). Watch the net to observe its performance and adjust to sample the surface properly.
8. Deploy trawl for 30 minutes.
9. Recover and secure trawl on boat. Record STOP DATA immediately (e.g., Latitude and Longitude, End time, Flowmeter End, Boat Speed End).
10. Record any notable details or deviations from protocols in Notes Section.

11. Using handheld sprayer with DI or MilliQ water, rinse material that is inside the Manta trawl into the netting attached to the trawl.
12. Using one of the vessel hoses, rinse the contents in the net down to the cod end, making sure that no water from the hose is used inside the net.
13. Fold the top portion of the cod end down and then slide the bottom up such that you are slowly turning the cod end inside out. Push the sample out of the cod end and into the large bowl. Invert the cod end and carefully rinse leftover sample from the inside of the cod end into the large bowl, using DI water squirt bottle.
14. Filter the contents of the bowl (sample plus rinsing water) through the Tyler sieve to reduce the volume of water in the sample. Using a minimal amount of DI water, transfer the sample from the sieve into a labeled sample jar. All sample containers will be purchased 'pre-cleaned' from ESS Vial, VWR or comparable laboratory equipment distributor.
15. Take photograph and video of each sample.
16. Add isopropyl alcohol to preserve the sample (Roughly 10% of liquid volume of sample).
17. Samples will be shipped to the analytical lab as soon as possible; however, there are no hold times for microplastic.

Microplastic Monitoring with Pump System (20 micron to 5mm):

1. Pump System samples will occur at selected monitoring sites.
1. All details listed on the *SF Microplastic Project Monitoring Event Field Form* will be completed and recorded for each sampling event during the project. For each sample, record Sample ID, Site ID, latitude and longitude at the beginning and end of sample collection, wind speed / direction, sample collection date and time, equipment type, water temperature, salinity, water current speed and direction, last rainfall, sea state, approximate wave height and direction, salinity, field staff, and weather condition, at a minimum.
2. Consistent Sample IDs will be recorded and clearly identified on each sample. Photographs will be taken of each sample and video will be recorded during each sampling event.
2. Pump Assembly – The Pump System includes an input filter with 5mm wire mesh screen, reusable filter holder with 47mm filter paper, manual hand pump and an output flow meter, connected by durable ¾-inch diameter tubing.
3. Prior to placing filters in the system, flush the system with 0.5 liters of DI water.
4. Stop vessel and stay in one general location during sampling.
5. Record appropriate data on Pump System Data Collection Form (e.g., Sample ID, Date, Wind Speed, Water Temp, Sea State, etc.). Reset flow meter or if the meter is calibrated to do continuous flow note meter value at start (and stop).
6. From the side of the vessel, place the pump system's input filter in the surface waters (within the top portion of the water). Record Start Time and Latitude and Longitude.
7. Filter approximately 10 liters of water through the system (Flow meter on the Pump System will record the amount of water passing through the system). This will take approximately 10-20 minutes, but will depend on how much natural and non-natural material is in the water.
8. Recover pump system on boat. Record STOP DATA immediately (e.g., Latitude and Longitude, End Time, Flowmeter End).

9. Record any notable details or deviations from protocols in Notes Section.
10. Remove filter and place in clean glass petri dish and cover with lid, when dry seal it with laboratory tape (all the way around).
11. Samples will be shipped as soon as possible; however, there is no hold time for microplastic analysis.

Nanoplastic Monitoring (<1 micron):

1. All details listed on the *SF Microplastic Project Monitoring Event Field Form* will be completed and recorded for each sample collected during the project. Consistent Sample IDs will be recorded and clearly identified on each sample. Photographs will be taken of each sample and video will be recorded during each sampling event.
2. Samples should be collected in 1-liter glass jars with foil-lined lids. Teflon caps or teflon cap liners should be used. Either technique lowers the risk of small plastic pieces breaking off and contaminating the sample. Pre-cleaned sample jars will be purchased from laboratory distributors.
3. For each sample, record Sample ID, Site ID, latitude and longitude at the beginning and end of sample collection, wind speed / direction, sample collection date and time, equipment type, water temperature, water current speed and direction, last rainfall, sea state, approximate wave height and direction, salinity, field staff, and weather condition.
4. Each sample should be collected from the downwind side of the vessel in the top 45 cm of the water.
5. Each 1-liter glass jar should be rinsed three times in situ with seawater at the time of sampling.
6. Samples should be taken immediately after the seawater rinse and capped underwater to reduce air exposure time. Minimizing air exposure time reduces potential airborne contamination of the sample. If the jar cannot be capped underwater, a stainless steel bucket will be used to fill the 1-liter glass jar, first rinsing the jar with sea water. Care should be taken not to freeze the samples lest the containers break.
7. Samples will be shipped as soon as possible; however, there are no hold times for nanoplastics.

Quality Assurance / Quality Control Sample Collection

All samples will be collected in glass sample containers that are purchased pre-cleaned from a laboratory equipment vendor. All equipment will be rinsed with rinsed with DI water prior to use in the field. Field blanks will be taken to assess the potential for the introduction of microplastic during sample collection and transport. All field blanks will accompany the field samples and will be shipped to the laboratory for analyses.

For Manta trawls, the field blanks will be collected by pouring at least 2 liters of DI water through the Manta trawl, and then transferring the contents of the cod end into a sieve, following the protocols described for sample collection. Four field blanks will be collected in the Bay and Sanctuaries (one wet season and one dry season). Four field duplicates will be collected in the Bay (two in summer and two in

winter) by retracing an existing trawl line. Two field duplicates in the Sanctuary (one in the dry season and one in the wet season) will also be collected.

For the pump system, a blank sample will be collected by pumping at four liters of DI water through the pump system. The filter from the blank pump sample will be shipped with the field samples. One field blank for the microplastic pump will be collected in Bay and Sanctuary per season. A field duplicate will be collected at one site during both wet and dry conditions. The duplicate will be collected directly after the first sample is collected using similar procedures.

For the nanoparticle blank sample, a clean 1L sample jar will be filled with DI water and shipped to the lab. One field blank for the nanoparticle samples will be collected per season. A duplicate sample will be collected at one site per season by collecting a second sample.

4. Sample Labeling

The sample ID system used for SF Bay and Marine sanctuary cruises for analytical samples is as follows:

YY-MATRIX-STA#-AGX

Where:

YY = Year (e.g., 17 = 2017)

MMP = Project (Moore Microplastic Project)

MATRIX = Matrix type. WM for water collected with a Manta trawl; WP20 for water collected with a 20 micron filter, WG for grab samples (for nanoparticle analysis);

STA# = Station ID, where the STA indicates site embayment and station number. Suisun Bay is abbreviated as SU; San Pablo Bay is SPB; Central Bay is CB; South Bay is SB; Lower South Bay is LSB; Greater Farallones National Marine Sanctuary is GFNMS; Cordell Bank National Marine Sanctuary is CBNMS; and Monterey Bay National Marine Sanctuary is MBNMS. This number is followed by station number.

AGX = Acronym for analyte group: Nano - NP; Micro - MP; Pump - PU

5. Sample Archive Strategy

Additional backup samples will not be collected at the time of sampling. Microplastic and nanoplastic samples will be archived at the partnering laboratories until the end of the project.

6. Field Equipment List

- Manta Trawl with 335 micron net
- 2-3 cod ends for Manta Trawl

- High tension nylon/polypropylene rope
- General Oceanics Flowmeter
- 355 micron Tyler Sieve
- 20 nm pump system
- Anemometer
- Water Quality Data Sondes
- Stainless steel bucket
- Screwdriver
- Large stainless steel or glass bowl
- Squirt bottles
- DI or MilliQ water for rinsing
- Tweezers
- Stainless steel spoon
- 1L sample bottles
- 500 ml sample jars/bottles
- Labels
- Permanent marker
- Rubber gloves
- Duct tape
- Preservative (Isopropyl or ethyl alcohol)
- Handheld sprayer
- SF Microplastic Project Monitoring Event Field Form
- Manta Trawl Data Collection Form
- Pump System Collection Form
- Nanoplastic Collection Form

7. Field Data Sheet

Surface Water Sampling in San Francisco Bay and Adjacent Sanctuaries

MMP Project Monitoring Event Field Form

Page ____ of _____

Field Conditions

Station ID	Region	Station Description	Latitude (N)	Longitude (W)	Date	Wind Speed/Direction	Sea State

Wave Direction/Height:	Current Strength/Direction:	Water Temp./Salinity/pH:	Last Rainfall (date/time):

Manta Trawl

Sample ID		Sampling Time	Latitude (N)	Longitude (W)	Flow Meter	Boat Speed/Heading	Distance Traveled
17MMP-WM-	Start:						
(STA_ID)	End:						
- MP	Media:		Preservative Added and Amount:	Notes:			
	Photo: Y / N						
	Video: Y / N						

Microplastic Pump

Sample ID		Sampling Time	Latitude (N)	Longitude (W)	Flow Meter	Number of Filters Used	Staff Initials
17MMP-WP20-	Start:						
(STA_ID)	End:						
- MP	Media:		Preservative Added and Amount:	Notes:			
	Photo: Y / N						
	Video: Y / N						

Nanoparticle Grab Sample

Sample ID		Sampling Time	Latitude (N)	Longitude (W)	Flow Meter		Staff Initials
17MMP-WG-	Start:						
(STA_ID)	End:						
- NP	Media:		Preservative Added and Amount:	Notes:			
	Photo: Y / N						

* include additional sheets as necessary for blanks and duplicates

Appendix B-2
Field Procedures for Sediment Collection

1. Sampling Design (See Sediment Cruise Plan for latest information)

Table B-2.1 Rationale and location of Sediment Samples (Summer 2017)

Embayment	Sediment Type	Site location/ ID	Target Latitude	Target Longitude	Sample already collected?	Rationale	Co-located site		
							Small fish site	Stormwater site	Effluent site
Central Bay	Ambient (2014)	Central Bay (CB001S)	37.87655	-122.3615	Yes	Background characterization			
Central Bay	Ambient (2014)	Central Bay (CB0073S)	37.84318	-122.39795	Yes	Background characterization			
Central Bay	Ambient (2014)	Central Bay (CB100S)	37.77725	-122.32939	Yes	Background characterization			
Central Bay	Ambient (2014)	Central Bay (CB133S)	37.83953	-122.3167	Yes	Background characterization			
Central Bay	Margins (2015)	Crab cove off of Alameda (CB04)	37.767583	-122.27775	Yes	Background characterization			
Central Bay	Margins (2015)	Richmond Marina Bay - just off of Vincent Park (CB10)	37.906683	-122.34667	Yes	Background characterization			
Central Bay	Margins (2015)	Just slightly northwest of Bay Bridge/IKEA (CB15)	37.8279	-122.303417	Yes	Urban creek - Temescal	Yes	Ashby spit - RMP sample ID	
Central Bay	Margins (2015)	East of Coast Guard island (CB24)	37.78635	-122.247483	Yes	Background characterization			
Central Bay	Margins (2015)	Albany Mudflat State Marine Park (CB30)	37.892833	-122.312	Yes	Background characterization			
Central Bay	Margins (2015)	San Leandro Bay - NE near East Creek Slough (CB32)	37.756633	-122.2204	Yes	Urban Creek - East Creek Slough	Yes	East Creek Sample-Line12FPG&E	
Central Bay	Margins (2015)	San Leandro Bay -SW (near Doolittle Dr -CB-48)	37.742767	-122.215517	Yes	Background characterization			
Central Bay	Margins (2015)	South of Oyster Point; very close to Colma Creek (CB37)	37.6414	-122.3945	Yes	Urban Creek - Colma Creek		Colma creek site	

Table B-2.1 Rationale and location of Sediment Samples (Summer 2017)									
Central Bay	Margins (2015)	Richardson Bay (north of Sausalito -CB39)	37.875833	-122.50725	Yes	Background characterization			
Central Bay	Margins (2015)	San Francisco -McCovey Cove (CB49)	37.776967	-122.388917	Yes	Urban Creek – Mission Creek			
South Bay	Ambient (2014)	South Bay (SB002S)	37.61039	-122.167	Yes	Background characterization		Alameda Creek?	
South Bay	Ambient (2014)	South Bay (SB004S)	37.60085	-122.21859	Yes	Background characterization			
South Bay	Ambient (2014)	South Bay (SB110S)	37.54753	-122.17277	Yes	Background characterization			
South Bay	Ambient (2014)	South Bay (SB111S)	37.69587	-122.22957	Yes	Background characterization			
South Bay	Margins (2017)	Westside - South of SFO runway (SB051)	37.6018	-122.36		Background characterization			
South Bay	Margins (2017)	Westside - Near Seal Slough (SB062)	37.5764	-122.27		Urban Creek (and golf course)		San Mateo?	
South Bay	Margins (2017)	Westside - Bair Island (SB077)	37.5452	-122.22		Wastewater (South Bay Systems Authority)			
South Bay	Margins (2017)	Westside - South of Bair Island – Redwood Creek (SB074)	37.5277	-122.18		Urban Creek			
South Bay	Margins (2017)	Westside - north of 84 - Ravenswood Slough (SB058)	37.4983	-122.16		Background characterization			
South Bay	Margins (2017)	Eastside – near Oro Loma (SB069)	37.6625	-122.18		Urban Creek - San Lorenzo			
South Bay	Margins (2017)	Eastside – Eden Landing (SB075)	37.6099	-122.16		Background characterization			
South Bay	Margins (2017)	Eastside – Alameda Creek (SB056)	37.5605	-122.13		Stormwater		Alameda Creek?	
Lower South Bay	Ambient (2014)	Lower South Bay (LSB002S)	37.47918	-122.07781	Yes	Background characterization			
Lower South Bay	Ambient (2014)	Lower South Bay (LSB004S)	37.49313	-122.08549	Yes	Background characterization			

Table B-2.1 Rationale and location of Sediment Samples (Summer 2017)									
Lower South Bay	Margins (2017)	Westside – north of San Francisquito (LSB11)	37.4716	-122.12		Background characterization			
Lower South Bay	Margins (2017)	Westside – near Palo Alto WWTP (LSB02)	37.4628	-122.11		Wastewater			Palo Alto WWTP
Lower South Bay	Margins (2017)	Westside – Hooks Point (LSB06)	37.4576	-122.09		Urban Creek / Background characterization			
Lower South Bay	Margins (2017)	Westside – Moffett Field (SOSL15)	37.4518	-122.06		Urban Creek – Stevens Creek			
Lower South Bay	Margins (2017)	North of Guadalupe (SOSL 16)	37.4576	-122.04		Urban Creek		Guadalupe Slough	
Lower South Bay	Margins (2017)	Coyote Creek (SOSL40)	37.4621	-122.02		Wastewater and urban creek	Yes	Coyote Creek	San Jose WWTP
Lower South Bay	Margins (2017)	Eastside near Mowry (LSB04)	37.4864	-122.07		Background characterization			
Lower South Bay	Margins (2017)	Don Edwards (LSB01)	37.4988	-122.08		Background characterization			
San Pablo Bay	Margins (2017)	China Camp (SPB126)	38.01959871	-122.4929642		Wastewater	Yes		
San Pablo Bay	Margins (2017)	Petaluma River (SPB15)	38.10835486	-122.4881351		Urban river	Yes		
San Pablo Bay	Margins (2017)	Sonoma Creek (SPB50)	38.14185161	-122.389608		Background characterization			
San Pablo Bay	Margins (2017)	Napa river (CAR42)	38.07369321	-122.2495516		Urban river			
San Pablo Bay	Margins (2017)	Hercules (SPB128)	38.0156487	-122.3002376		Background characterization			
Suisun Bay	Margins (2017)	Contra Costa WWTP (SUB53)	38.04409028	-122.0969033		Wastewater / Urban creek – Pacheco Creek			Contra Costa
Suisun Bay	Margins (2017)	Montezuma Slough (SUB52)	38.13620888	-122.0349853		Baseline characterization			
Suisun Bay	Margins (2017)	Point Edith Wildlife (SUB16)	38.0524	-122.07693		Background characterization			
Tomales Bay	Margins (2017)	Near Long Cove Beach	38.16073	-122.8985		Reference			
Tomales Bay	Margins (2017)	South End of Bay	38.09084	-122.83581		Reference	Yes		

Table B-2.1 Rationale and location of Sediment Samples (Summer 2017)									
Tomales Bay	Margins (2017)	Near Walker Creek	38.20926	-122.92915		Reference	Yes		

2. Cruise Schedule

Table B-2.2. Anticipated Cruise Schedule for 2017 Microplastic Sediment Sampling San Francisco and Tomales Bays

Date	Time	Vessel	Activity
June - August	TBD	Boston Whaler	San Francisco Bay. Sites will be scheduled dependent on tides and RMP margin sediment collection. As such, the sampling will occur over the summer of 2017.
June - August	TBD	TBD	Tomales Bay

3. Field Sampling Methods and Sample Processing

Sediment will be collected from the top 5 cm of the surface. Sediment station coordinates listed in Table B-2.1. At each site, a 500 ml sample for University of Toronto and a 750 ml sample for University of Michigan will be collected. Field observations should also be noted for each site (e.g., sediment color, sediment odor, sediment composition, etc.). Sediment samples will be collected and processed following the procedures in the following subsections. Finalized sampling methods for sediment are outlined in the 2017 Bay Margins Sediment Study Cruise Plan and 2017 Cruise Plan for Microplastics in Sediment in San Francisco Bay (North)/Tomales Bay and Fish in San Francisco Bay.

Sample Equipment and Cleaning

Intertidal and subtidal zone sampling for Bay margin sites will be conducted from an 18' Boston Whaler equipped with frame and hydraulics for deploying either a 0.05 m^2 or a 0.1 m^2 modified Van Veen sediment grab. The grab is constructed entirely of stainless steel and the jaws and doors are coated with Kynar™ to improve chemical inertness. The scoop and bucket used to remove and composite sediments are metal; avoiding plastic tools reduces potential sample contamination via collection.

All sampling and handling will be conducted using clean techniques. Prior to sampling, all sampling equipment will be thoroughly cleaned. Equipment that is pre-cleaned includes the Van Veen grab, sample scoops, and wash bottles. The Van Veen grab will be cleaned with detergent and pressure washed at the lab. Other equipment is washed, with a detergent and deionized water solution, and rinsed three times with deionized water in lab pre-cleaning, which can be substituted by ambient water in the field. Equipment is next rinsed with 1.0 % solution of hydrochloric acid (or equivalent), followed by a rinse with methanol, followed by another set of three rinses with deionized water (or ambient water in the field). All equipment besides the Van Veen grab is stored in clean Ziploc™ bags until used in the field. Equipment used at different sampling stations will be re-cleaned by rinsing in ambient water in the field between uses. All sample containers will be purchased 'pre-cleaned' from ESS Vial or VWR.

Sampling personnel will wear nitrile gloves whenever taking or processing samples to avoid contact contamination (and for personal protection). In addition, airborne contamination is avoided by keeping sample containers, and sample scoops. Fabrics that shed plastic fibers should be avoided (e.g., fleece). If possible, wear natural fibers or a wind/rain jacket when collecting samples.

Sediment Collection Protocol

The frame at the side of the vessel will be used for deploying the Van Veen grab. The quality of grab samples will be ensured by requiring each sample to satisfy a set of criteria concerning the depth of penetration and disturbance of the sediment within the grab. In this way, each sample will normally contain the top 5-cm of sediment within the area of the grab jaws. Grab samples will be rejected for the following conditions:

- There is a rock or shell fragment wedged between the jaws of the grab allowing the sample to wash out.
- The sample surface is significantly disturbed.
- The sample is uneven from side to side, indicating that the grab was tilted when it penetrated the sediment.
- The surface of the sample is in contact with the top doors of the grab, indicating over-penetration of the grab and possible loss of material around the doors.

The total number of grabs taken will be recorded by field personnel on the field datasheets (see Section 7 below). Sediment samples for microplastic will be collected to a depth of 5 cm and sediment grabs will be taken until at least 2 L of sediment is collected, and 4.5 L of sediment for sites with field duplicates. Sediment grabs showing prior disturbance (e.g., from immediate/recent prior grabs at the same site) should be retaken from an undisturbed area. Excess sediment should be kept on the boat until collection for the site is done where possible, unless there is sufficient flow to ensure that discarded sediment is not redistributed to areas later collected in subsequent grabs for the same site (e.g., by always heading up the current for later grabs).

Sediment samples will be collected by filling the containers in the field. Three sediment samples will be collected at each site: 500 ml for microplastic analyses (University of Toronto); 1-L for nanoplastic analyses (University of Michigan); and 1-L for short term archive.

All sediment sample bottles should be filled to around 75% of total capacity unless otherwise specified, to allow room for expansion on freezing, as needed. After placing the sediment into the container, a layer of aluminum foil will be placed on the top of the glass jar to avoid cross-contaminating the sample with any plastics that may be associated with the lid. The dull side of foil should be positioned downward toward the sediment sample. Sample containers may be bagged in ziploc to avoid contamination and then bubble wrap bagged or placed in their original shipping box with cardboard separators to reduce potential container breakage. Samples will be shipped frozen to the laboratories. There are no hold times for microplastic or nanoplastic.

QA/QC Sample Collection

Field duplicates will be collected at three sites by collecting a larger sample volume (1-Liter). The laboratory will subsample and analyze.

Field blanks will be collected at four sites in the field using DI water. Water will be poured across the sampling equipment into the sample container in the field and the bottle covered with foil (dull side down towards the sample) and then sealed. These field blanks will remain with the samples and will be processed in the laboratory.

4. Sample Archive Strategy

At every site, a one-liter sample bottle will be filled with sediment and saved for short-term archive in the event that a sample is lost during transport or compromised in the laboratory. The sample will be retained in the archives until all microplastic results have been reported to SFEI. Prior to discarding the archive samples, CCR will contact SFEI.

5. Sample Labeling

The sample ID system used for SF Bay sediment cruises for analytical samples is as follows:
YYMMP-MATRIX-STA#-AGX

Where:

YY = Last 2 digits of Year (e.g., 17 = 2017)

MMP = Project (Moore Microplastic Project)

MATRIX = Matrix type. S for sediment.

STA# = Station ID where the STA indicates site embayment and station number. Suisun Bay is abbreviated as SU; San Pablo Bay is SPB; Central Bay is CB; South Bay is SB; Lower South Bay is LSB; and Tomales Bay is TB. This number is followed by station number.

AGX = Acronym for analyte group: Nano - NP; Micro - MP; MPA - Archive

6. Field Equipment List

- Anemometer
- Water Quality Data Sondes
- Stainless steel bucket
- Screwdriver
- Large stainless steel or glass bowl
- Stainless steel spoon
- 1L sample glass collection bottles
- 500 ml sample glass collection bottles
- Labels
- Permanent marker
- Rubber gloves
- Duct tape
- DI or MilliQ water

7. Field Sheet

Sediment Sampling in San Francisco Bay

MMP Project Monitoring Event Field Form

Station Information		
Station ID:	Date:	Time On Station:
Station Coordinates (decimal degrees, 6 decimal places)	Latitude North	Longitude West

Field Observations	
Wave Height (ft):	Wind speed (circle one): Calm Breezy Strong
Filenames of Any Photos Taken:	General Comments:
Grab 1 - Sediment Description (circle one) Sand Silt Mud Rocky/Shell Hardpan	Grab 2 - Sediment Description Sand Silt Mud Rocky/Shell Hardpan
Grab 3 - Sediment Description Sand Silt Mud Rocky/Shell Hardpan	Grab 4 - Sediment Description Sand Silt Mud Rocky/Shell Hardpan

Samples Filled

Sediment Volume	# per Site	Collection and Handling	Sample ID 2017MMP-S-STA#-AGX	Analysis or Purpose	Date Filled/ Frozen
1-L Glass	1 (or2)	Homogenize in lab. Fill at least 500 mL, and no more than 3/4. Freeze.		Microplastics by Univ of Toronto	
1-L Glass	1	Homogenize in lab. Fill about 750 mL. Freeze.		Nanoplastic by Univ. of Michigan	
1-L Glass	1	Homogenize in lab. Fill at least 500 mL, and no more than 3/4. Freeze.		Short term archive	

Appendix B-3
Field Procedures for Prey Fish Collection

1. Sampling Design (see the Cruise Plan for Sediment and Fish for latest information)

As described in the body of the Sampling and Analysis Plan, six sites in San Francisco Bay have been targeted to collect anchovy (10 individual fish) and Mississippi silversides or topsmelt (10 individual fish). Two sites are located in a reference region, Tomales Bay near the Point Reyes National Seashore (Table 5-1).

Table B-3.1 Location of prey fish sample sites and correlation with other samples collected

Site	Location	Target Latitude	Target Longitude	Sediment site	Stormwater site	Wastewater site	Ambient water site
Tomales Bay (Reference)	Southern portion of Bay	38.09084	-122.83581	Yes			
Tomales Bay (Reference)	Near Walker Creek (North end)	38.20926	-122.92915	Yes			
Central Bay	Just slightly northwest of Bay Bridge/IKEA (CB15)	37.82928	-122.30548	Near CB-15	AC-2017-Line12A		Yes
Central Bay	San Leandro Bay - NE near East Creek Slough (CB32)	37.75787	-122.30548	Near CB-32	AC-2017- Line12F		Yes
Lower South Bay	Near Hooks Point	37.457572	-122.092072	Yes		Palo Alto	Yes
Lower South Bay	Near Guadalupe River	37.45490	-122.04529	Yes	Guadalupe River (upstream)	San Jose	Yes
North Bay	San Pablo Bay near Petaluma River	38.10327	-122.48163	Yes			
North Bay	San Pablo Bay near China Camp	38.02508	-122.48969	Yes			

2. Cruise Schedule

Small prey fish will be collected in concert with the margin sediment sites during the summer of 2017. The exact dates of collection need to be determined and will be a function of sampling site conditions (e.g., tides) and logistics.

3. Field Sampling Methods and Sample Processing

Prey fish will be collected at six sites within the Bay and two sites located in Tomales Bay, a reference location approximately 45 km north of San Francisco Bay.

At each sample site, a minimum of 20 fish will be collected (10 - topsmelt and/or Mississippi silverside and 10 - anchovy). Any additional fish collected should be archived in case of loss of samples during shipment or other unforeseen conditions. At two sites (Lower South Bay – Guadalupe river and Tomales Bay – southern portion), 10 additional prey fish (topsmelt or Mississippi silverside) will be collected for nanoplastic analyses.

Field Parameters

Field observations should also be noted for each site (e.g., wind speed, wave height, weather, etc.). Sites will sometimes partially overlap with sediment sites but may slightly differ depending on where the fish are present. Fish samples will be collected and processed following the procedures in the following subsections. The field sampling crew (Coastal Conservation and Research (CCR)) is in the process of developing a field collection sheet.

Sample Equipment and Collection

Fish will be collected by Coastal Conservation and Research using trawl, gill, cast and tide nets. The coordinates of the actual sampling site will be determined using a handheld or shipboard global positioning system (GPS) and reported on field sheets provided by CCR. Other pertinent information will also be recorded, including the sampling method, device, depth, and descriptive location. For samples collected over an area, an extent or rough polygon of the area of capture will be reported.

The analytical laboratory will initially focus on the gut contents of the prey fish; however, fillets and skins may be monitored for microplastic and/or other contaminants, so care should be taken when handling and processing the samples prior to analysis.

Once they are caught, fish will be placed on a pre-cleaned measuring board and the smallest and largest fish will be measured for total length to provide a size range. Length measurements will be conducted on a fish measuring board that does not require calibration. Individual fish will be measured for fork length and total length and weighed in the laboratory at University of Toronto. When possible, sex, parasites, and body anomalies will be noted on the laboratory sheet.

While in the field, fish will be individually wrapped in aluminum foil (dull side facing toward the fish), placed in a clean, labeled bag, and then frozen on dry ice immediately. CCR will check on the samples periodically to ensure that they are appropriately protected and there is sufficient dry ice.

At the end of each sampling week, CCR will transport the samples on dry ice to a -20C storage freezer. CCR will arrange for a courier to transport the prey fish samples on dry ice to University of Toronto and University of Michigan where they will then be stored at -20C until processing. There is no hold time for microplastic analyses. All samples will be accompanied by a chain of custody form (COC). The COC form will include the sample unique ID, site name, collection date, sample type, analysis required, and other remarks. For each set of samples being shipped to a laboratory or archive, CCR will initiate a COC form and include the original form with the sample shipment, and provide a digital copy/scan of the form to SFEI data management team at the time of the shipment. Chain of custody records will be maintained throughout the course of the sampling effort. In addition, all field sheets will be scanned and sent to SFEI.

4. Sample Archive Strategy

If additional fish are caught above the desired target quantity, they will be archived and the species type and number annotated on the field sheets. The fish will be processed similarly to the samples that will be shipped to University of Toronto; however, they will be maintained in a short term archive at Moss Landing Marine Laboratories. This is to ensure against sample loss during shipment or compromised samples during laboratory processing. Archives will be maintained until the project is completed. CCR will check with SFEI prior to discarding sample archives.

5. Sample Labeling

The sample ID system used for San Francisco Bay and Tomales Bay cruises for analytical samples is as follows:

YYMMP-SP-STA#-AGX

Where:

YY = Year (e.g., 17 = 2-17)

MMP = Project (Moore Microplastic Project)

SP= Species. TS = Topsmelt. MS = Mississippi silverside. AN = Anchovy.

STA# = Station ID where the STA indicates site embayment and station number. Suisun Bay is abbreviated as SU; San Pablo Bay is SPB; Central Bay is CB; South Bay is SB; Lower South Bay is LSB; and Tomales Bay is TB. This number is followed by station number.

AGX = Acronym for analyte group: Micro - MP; MPA – Archive; NP - nanoparticle

6. Field Equipment List and Field Sheet

The subcontractor, CCR, will develop a field equipment list and field data sheets.

Appendix B-4
Field Procedures for Wastewater Collection

1. Sampling Design

As described in the body of the Sampling and Analysis Plan, eight facilities will be targeted to collect samples over the course of 24 hours (Table 6-1). These facilities will be sampled twice to evaluate the variation in effluent concentrations collected.

2. Sampling Schedule

Samples will be collected in the Fall 2017.

3. Field Sampling Methods and Processing Protocol

Sample Collection Methods

SFEI staff will work with each wastewater treatment facility to determine how best to collect a 24-hour composite. If effluent is available from an easily accessible port, SFEI staff will use the port to divert flow across the 355 micron and 125 micron sieves. To avoid clogging the screens, we may use a 5 mm and 1 mm sieve as a pre-filter. If effluent is less accessible (e.g., must be obtained from a confined space), SFEI will work facility personnel to have them collect the sample.

The samples will be taken over a 24-hour period. The sampling will occur Tuesday through Friday to avoid variation that may occur on the weekend. It will be necessary to determine flow rate, preferably measuring flow throughout the 24-hour period using a flow monitor at the point of discharge. Should such a device not be available, flow can be estimated by measuring how long it takes to fill a 10 liter bucket with water; repeat a total of three times. At minimum, flow must be measured at the start of the collection and at the end of collection.

Samples will be collected in the following manner:

1. All details listed on the wastewater field form (see Section 7 of Appendix B-4 below) will be completed and recorded for each sample collected during the project. Consistent Sample IDs will be recorded and clearly identified on each sample. When possible, photographs will be taken of each sample.
2. Sieve Assembly – Prior to being in the field, the 355 micron and 125 micron sieves should be washed thoroughly and rinsed at least three times before assembled. The 355 micron sieve should be placed on top of the 125 micron sieve and secured. Place aluminum foil dull side inward around the top sieve with an opening for the flow of wastewater. The foil will act as a barrier so that water does not spill out during field work.
3. At the wastewater site, reassess the means of calculating flow, based on prior discussion with wastewater staff. In the event that a flow monitoring device is not present, time how long it takes to fill a 10 liter bucket with water; repeat a total of three times.

4. Place sieves in the effluent flow for 24 hours. Assure that aluminum foil acts to capture all water flowing from the sample port. Discuss any procedures that wastewater staff may need to conduct while project staff are absent, such as measuring flow.
5. After sampling is complete, time how long it takes to fill a 10 liter bucket with water; repeat a total of three times.
6. The sieves may be processed on-site or transported back to the SFEI lab and stored wrapped in foil until scientists prepare the sample for transportation.
7. Using squeeze bottle with deionized water, wash microplastic in each sieve into two different glass sample jars (500 ml). It is helpful to squirt all material (microplastic and natural) onto one side of the sieve and use a steady stream of water to wash the material into the sample jar. Spoons and tweezers may also be useful in transferring the material to the jar.
8. Take a photograph and video of each sample (with sample number clearly identified).
9. If deemed necessarily, isopropyl alcohol may be used as a preservative.
10. Sample bottles should be put into ziplock bags, bubble-wrapped and shipped on cold ice packs. Chain of custody forms will be filled out and placed in the coolers. A copy of COC will be maintained on-site. Upon receipt, the COC will be filed with the Data Manager.
11. There is no hold time for microplastic analyses.

QA/QC Sample Collection

A field duplicate will be collected at one site using a Y splitter on the sampling port to split the flow into two streams. Two different sieve stacks will be used for 24 hours to obtain composite samples. A field blank will be collected at one site. The field blank will be collected by setting up a sieve stack in the vicinity of the field sampling sieve set. The field blank sieves will remain uncovered for the duration of the 24-hour sampling event. The purpose of the field blank will be to assess contamination from the deposition of airborne microparticles during the 24-hour sampling event. The blank sieves will be processed in the same manner as the sampling sieves.

4. Sample Archive Strategy

Additional backup samples will not be collected at the time of sampling. Microplastic samples will be archived at the partnering laboratory until the end of the project.

5. Sample Labeling

The sample ID system used for wastewater for analytical samples is as follows:

YYYYMMDDMMP-MATRIX-STA#-AGX

Where:

YYYY = Year (e.g., 2017), month (MM) and day (DD)

MMP = Project (Moore Microplastic Project)

MATRIX = Matrix type. Eff for effluent.

STA# = Station ID where the STA indicates site the acronym for the wastewater treatment facility.

AGX = Acronym for analyte group: Micro - MP

6. Field Equipment List

Microplastic Monitoring (125 micron and above):

- Duct tape
- 0.355 and 0.125 Tyler Sieves
- Aluminum foil
- Screwdriver
- 5-Gallon bucket to measure discharge
- Glass sample bottles (500 ml)
- Squirt bottles
- Tweezers
- Stainless steel spoon
- Labels
- Permanent marker
- Nitrile gloves
- Preservative (Isopropyl or ethyl alcohol)
- SF Microplastic Project Monitoring Event Field Form
- Wastewater Collection Form
- DI or Milli Q water or if available use facility's DI/Milli Q water

7. Field Sheet

Wastewater Sampling at San Francisco Bay WWTPs

MMP Project Monitoring Event Field Form

Wastewater Treatment Facility:		
Sample ID:	Start Date:	Start day of week:
Sample personnel/ WWTP Staff assisting:	Start time:	Stop time:
Field Observations:		
Sample port location (sink, exterior pipe etc.)	General Comments:	
Filenames of Any Photos Taken:		
Flow Measurement		
Method to determine flow (bucket/ time; meter, etc):	**Meter** Reading at start:	Reading at finish:
If using bucket, volume:	Start time:	Stop time:
If using bucket, volume:	Start time:	Stop time:
If using bucket, volume:	Start time:	Stop time:

Appendix B-5
Field Procedures for Stormwater Collection

1. Sampling Design

Table B-5.1 Rationale and location of Stormwater Microplastic Samples in San Francisco Bay (Winter 2017 and 2018)

Monitoring Sites[1]	RMP Site Name	Location	Priority	Latitude	Longitude	Size of Watershed (km2)	Rationale for site selection	Collected?
MMP-Storm-CB-Ash	Outfall to Bay just south of Ashby Spit	Central Bay	Priority 1	37.84582	-122.29915	7.15	RMP site, Urban (Commercial / Residential)	
MMP-Storm-CB-Line12A	Line12AatShellmoundStPedestrianBr	Central Bay	Priority 1	37.83429	-122.29349	10.48	RMP site, Urban (Commercial / Residential)	
MMP-Storm-CB-Line12F	Line12FbelowPGEstation	Central Bay	Priority 1	37.76218	-122.21431	10.18	RMP site, Urban (Commercial / Residential)	Yes
MMP-Storm-CB-Col12H	Line12HatColiseumWay	Central Bay	Priority 2	37.76238	-122.21217	0.97	RMP site, Only use if Coliseum 12K is not available. Low priority because of small drainage area	
MMP-Storm-CB-Col12I	Line12IatColiseumWay	Central Bay	Priority 2	37.75998	-122.2102	3.41	RMP site, Only use if Coliseum 12K is not available.	
MMP-Storm-CB-Col12J	Line12Jatmouthto12K	Central Bay	Priority 2	37.75474	-122.20136	8.81	RMP site, Only use if Coliseum 12K is not available	Yes
MMP-Storm-CB-Col12K	Line12KatColiseumEntrance	Central Bay	Priority 1	37.75446	-122.20431	16.4	RMP site, Site is near bay and includes commercial, residential and industrial	Yes
MMP-Storm-CB-Col12M	Line12MatColiseumWay	Central Bay	Priority 2	37.74689	-122.20069	5.3	RMP site, Only use if Coliseum 12K is not available	
MMP-Storm-CB-Meek	MeekerSloughatRegattaBlvd	Central Bay	Priority 1	37.42985	-121.90913	7.34	RMP site, Mixed residential, Drains into inner harbor in Oakland	
MMP-Storm-CB-SFPUC	*Not RMP site*	Central Bay	Priority 1	TBD	TBD	n/a	Major urban area, SFPUC	
MMP-Storm-SB-Coyote	*Not RMP site*	South Bay	Priority 1	37.385817	-122.909494	828[2]	303d listed for trash, Part of Tracking CA Trash Project, Major Tributary	
MMP-Storm-SB-SM	*Not RMP site*	South Bay	Priority 1	37.570019	-122.318567	360[2]	303d listed for trash, Part of Tracking CA Trash Project, Major Tributary	
MMP-Storm-SB-SFC	SanFrancisquitoCreek atUniversityAve	South Bay	Priority 1	37.4579	-122.14214	81.76	RMP site, Residential and Commercial	
MMP-Storm-SB-Colma1	Colma Ck at Linden	South Bay	Priority 1	37.650205	-122.411865	27.5	RMP site, 303d listed for trash, Part of Tracking CA Trash Project, Major Tributary	Yes

Table B-5.1 Rationale and location of Stormwater Microplastic Samples in San Francisco Bay (Winter 2017 and 2018)

MMP-Storm-SB-Colma2	Outfall to Colma Ck on service rd nr Littlefield Ave. (359A)	South Bay	Priority 1	37.6429	-122.39677	n/a	RMP site, Outfall to Colma Creek	
MMP-Storm-SB-Colma3	Outfall to Colma Ck on service rd nr Harbor Way and Littlefield Ave. (1001C)	South Bay	Priority 1	37.64309	-122.3993	n/a	RMP site, Outfall to Colma Creek	
MMP-Storm-SB-RedCity1	Price Track PS (336A)	South Bay	Priority 1	37.49236	-122.22747	0.27	RMP site	
MMP-Storm-SB-RedCity2	Outfall at Blomquist and E Bayshore Rd (407A)	South Bay	Priority 1	37.49317	-122.21292	n/a	RMP site, Storm Drain	
MMP-Storm-CB-Alameda	Line 5A (Alameda Ck) at EBRPD Bridge at Quarry Lakes	South Bay	Priority 1	37.56666	-122.00142	911.37	RMP site (Line 5A (Alameda Ck), Open space, Large drainage area	
MMP-Storm-SB-Dry	Line5LDryCkatAlvaradoNilesRd	South Bay	Priority 2	37.59086	-122.03432	25.33	RMP site, Open space, Medium drainage area	
MMP-Storm-LSB-Matadero	MataderoCkatCowperSt	Lower SB	Priority 1	37.42918	-122.12866	25.27	RMP site, Mixed use	
MMP-Storm-LSB-SanJose1	E. Gish Rd SD 066GAC550	Lower SB	Priority 2	37.36632	-121.90203	0.44	RMP site, small drainage area	
MMP-Storm-LSB-SanJose2	North Fourth St SD 066GAC550B	Lower SB	Priority 2	37.361956	-121.905349	n/a	RMP Site, Storm Drain	
MMP-Storm-LSB-SanJose3	Rosemary St SD 066GAC550C	Lower SB	Priority 2	37.361175	-121.905938	n/a	RMP Site, Storm Drain	
MMP-Storm-LSB-Guad850	GuadalupeRoutfall066GAC850A	Lower SB	Priority 2	37.35469	-121.91279	3.35	RMP site, Industrial, commercial, residential	
MMP-Storm-LSB-Guad900	GuadalupeRoutfall066GAC900A	Lower SB	Priority 2	37.35392	-121.91223	0.17	RMP site, Industrial, commercial, residential, Small drainage area	
MMP-Storm-LSB-Guad010	Guadalupe R outfall 067GAC010A	Lower SB	Priority 2	37.35209	-121.91153	n/a	RMP site, Industrial, commercial, residential, Small drainage area	
MMP-Storm-LSB-Guad075	Guadalupe R outfall 067GAC075A	Lower SB	Priority 2	37.34937	-121.90983	n/a	RMP site, Industrial, commercial, residential, Small drainage area	
MMP-Storm-LSB-Guad150	Guadalupe R outfall 067GAC150A	Lower SB	Priority 2	37.34588	-121.90649	n/a	RMP site, Industrial, commercial, residential, Small drainage area	
MMP-Storm-LSB-Guad	Guadalupe River	Lower SB	Priority 1	37.373599	-121.932679		RMP site near Highway 101	Yes
MMP-Storm-LSB-SFC	San Francisquito Ck. at University Ave.	Lower SB	Priority 1	37.4579	-122.14214	81.76	RMP site, large drainage, mixed use site	
MMP-Storm-NB-EAntioch	EastAntiochnrTrembath	Suisun Bay	Priority 2	38.00333	-121.78106	5.25	RMP site	

Table B-5.1 Rationale and location of Stormwater Microplastic Samples in San Francisco Bay (Winter 2017 and 2018)

MMP-Storm-NB-LittleBull	LittleBullValley	Suisun Bay	Priority 2	38.037187	-122.179749	0.33	RMP site, Small drainage area	
MMP-Storm-NB-MtDiab	MtDiabloCkPortChicagoHwy	Suisun Bay/Inland	Priority 2	38.01876	-122.02688	80.1	RMP site, Inland	
MMP-Storm-NB-Kirker	Kirker Ck at Pittsburg Antioch Hwy and Verne Roberts Cir	Suisun Bay/Inland	Priority 2	38.01275	-121.84345	n/a	RMP site	
MMP-Storm-NB-Refugio	RefugioCkatTsushimaSt	San Pablo Bay	Priority 1	38.01775	-122.2771	10.73	RMP site, Open space	Yes
MMP-Storm-NB-Rodeo	RodeoCreekatSeacliffCtPedestrianBr	San Pablo Bay	Priority 1	38.016056	-122.253677	23.41	RMP site, Open space	Yes

1 – Sites are based on 2016/2017 RMP Small Tributary Monitoring Sites and may change in 2018

2 – Estimated drainage area

3 – A field duplicate will be taken at one site using two sets of sieves, with samples taken in series (the field duplicate sampled immediately following the field sample). A field blank will be collected at one site.

2. Sample Schedule

Table B-5.2. Anticipated Schedule for 2017/2018 Stormwater Sampling in Bay

Date	Activity
Early 2017 and Fall 2017	Work with SFEI's RMP field team to develop and test protocols to monitor stormwater.
Winter 2017/2018	Work with SFEI's RMP field team to sample at least seven monitoring sites during wet weather.

3. Field Sampling Methods and Sample Processing

Microplastic Monitoring with ISCO (125 micron and above):

1. All details listed on the *MMP Project Monitoring Event Field Form* will be completed and recorded for each sample collected during the project. Consistent Sample IDs will be recorded and clearly identified on each sample. When possible, photographs will be taken of each sample and video will be recorded during each sampling event.
2. ISCO Sampler Assembly – The ISCO Sampler should be assembled and a suction line (⅜ - inch diameter tube) should run through the system. One end of the suction line should be long enough to reach the bottom of the receiving water or stormwater channel that is being analyzed.
3. Sieve Assembly – Prior to being in the field, the 355 micron and 125 micron sieves should be washed thoroughly and rinsed at least three times with DI water before assembled. The 355 micron sieve should be placed on top of the 125 micron sieve and secured. Place tin foil (dull side towards sieves) around the top sieve with an opening for the tube to fit in. The foil will act as a barrier so that water does not splash particles out during the collection of the sample.
4. At the stormwater site, the ISCO sampler and other equipment will be brought to sample site and placed on a bridgeway above the site. The long end of the tubing should be attached to the extendable pole with duct tape. This should be attached in such a way that the end of the tube is at the end of the pole. The short end of the tube should be placed over the sieves with foil wrapped around it. The end of the suction line should only be touched using gloves.
5. Sampling should begin as soon as rainfall begins and the storm hydrograph begins to rise. Priority should be made for microplastic samples to be collected at the beginning of the storm. Sampling should continue throughout the storm, as many times as is feasible throughout a storm or evenly spread out if the storm will last multiple hours. The number of sips throughout the storm will be determined in the field based on the storm system and likely duration.
6. For each sip, at least 3-5 gallons (or more) of water should be pumped through the sieve. This is done by starting the ISCO sampler and lowering the end of the tubing attached to the expandable pole into the waterway, collecting water from near the base of the stream channel to the surface. Water will be pumped across the same sieves multiple times throughout the storm, resulting in a composite sample of at least 30-50 gallons of water. In between sips, the sieves will be covered and placed in dedicated coolers to avoid cross-contamination.
7. The sieves should be carefully transported back to the SFEI lab and processed as soon as possible.
8. Using squeeze bottle with deionized water, wash microplastics in each sieve into two different glass sample jars (500 ml). It is helpful to squirt all material (microplastics and natural) onto one side of the sieve and use a steady stream of water to wash the material into the sample jar. Spoons and tweezers may also be useful in transferring the material to the jar.
9. If possible, take photographs of samples that contain visible plastic pollution (make sure to include sample number in photograph).
10. If necessary, add isopropyl alcohol to preserve the sample; to date, this has not been needed.

11. Sample bottles should be put into ziplock bags, bubble-wrapped and shipped on cold ice packs.
12. There is no hold time for microplastic analyses.

Microplastic Monitoring with Stainless Steel Bucket on USGS Type A Crane (125 micron and above):

Where site logistics prevent use of the ISCO sampler, an alternate method may be employed:

1. All details listed on the *MMP Project Monitoring Event Field Form* will be completed and recorded for each sample collected during the project. Consistent Sample IDs will be recorded and clearly identified on each sample. When possible, photographs will be taken of each sample and video will be recorded during each sampling event.
2. Sieve assembly – Prior to being in the field, the 355 micron and 125 micron sieves should be washed thoroughly and rinsed at least three times with DI water before assembled. The 355um sieve should be placed on top of the 125 micron sieve and secured. If the sieves do not connect tightly, use tape to secure the sieves together. Place tin foil (dull side towards sieves) around the top sieve with an opening for the tube to fit in. The foil will act as a barrier so that water does not spill out during field work.
3. Set up USGS Type A Crane with 4-wheel truck (and weights) on downstream site of bridgeway.
4. Sampling should begin as soon as rainfall begins and the storm hydrograph begins to rise. Priority should be made for microplastic samples to be collected at the beginning of the storm. Sampling should continue throughout the storm, as many times as is feasible throughout a storm or evenly spread out if the storm will last multiple hours. The number of sips throughout the storm will be decided in the field based on the storm intensity and duration.
5. Attach 3-gallon bucket to metal line on USGS Type A Crane. Lower bucket into receiving water, focusing on collecting sample from surface waters.
6. Bring sample up to bridgeway and pour all water through the sieves. The sieves are held above a 5-gallon pail that is used to measure total volume passed through sieves.
7. Repeat each time sip is collected. This means that water will be passed through the same sieves multiple times throughout the storm, resulting in a composite sample of at least 30-50 gallons of water. Between sips, the sieves will be covered and stored in dedicated coolers to avoid cross contamination.
8. The sieves will be transported back to the SFEI lab and processed as soon as possible.
9. Using squeeze bottle with deionized water, wash microplastics in each sieve into two different glass sample jars (500 ml). It is helpful to squirt all material (microplastics and natural) onto one side of the sieve and use a steady stream of water to wash the material into the sample jar. Spoons and tweezers may also be useful in transferring the material to the jar.
10. If possible, take photographs of samples that contain visible plastic pollution (make sure to include sample number in photograph).
11. Add isopropyl alcohol to preserve the sample (Roughly 10% of liquid volume of sample)
12. Sample bottles should be put into ziplock bags, bubble-wrapped and shipped on cold ice packs.
13. There is no hold time for microplastic analyses.

Quality Assurance / Quality Control Sample Collection

A field duplicate will be taken at one site using two sets of sieves; 3-gallon sips will be taken in series with the duplicate sample following the field sample (i.e., 3-gallon sip that is pumped across first sieve set; second 3-gallon sip that is pumped across the second set). A field blank will be collected at one site. The field blank will be collected by opening a clean set of sieves and exposing them to the environment for the duration of the sample collection The field blank will be covered and placed with the sample sieves in between the collection events. For each set of samples being shipped to a laboratory, 5 Gyres will initiate a COC form (see Appendix D)**,** include the original form with the sample shipment, and provide a copy/scan of the form to SFEI at the time of the shipment.

4. Sample Labeling

The sample ID system used for the Stormwater Monitoring samples is as follows:

YYYYMMDD-MMP-MATRIX-STA#-AGX

Where:

YYYY = Year (e.g., 2017), month (MM) and day (DD)

MMP = Project (Moore Microplastic Project)

MATRIX = Storm – Stormwater-SW

STA# = Station ID where the STA indicates site embayment and station number. STA# will refer to RMP site name where possible. This number is followed by station number.

AGX = Acronym for analyte group: Micro - MP

Sample labels should also include RMP codes if it is an RMP site so that the information can be cross-referenced.

5. Sample Archive Strategy

Additional backup samples will not be collected at the time of sampling. Microplastic samples will be archived at the partnering laboratory until the end of the project. Lower priority samples (i.e. Priority 2) will be retained by the lab in the event that an insufficient number of Priority 1 samples are collected.

6. Field Equipment List

Microplastic Monitoring with ISCO (125 micron and above):

- Extendable pole
- Duct tape
- ISCO Sampler with ⅜ - inch diameter suction line (20-feet in length)
- 0.355 and 0.125 Tyler Sieves

- Aluminum foil
- Screwdriver
- 5-Gallon bucket to measure discharge
- Labels
- Permanent marker
- Nitrile gloves
- SF Microplastic Project Monitoring Event Field Form
- Stormwater Collection Form

***Microplastic Monitoring with Stainless Steel Bucket (125 micron and above)*:**

- Same list as above with the addition of a
  3-Gallon metal pail

7. Field Sheet

WY 2017 Storm Sampling Field Sheet - WQ only site

Staff:

Sampling Location:

Date(s):

BottleID:	Analyte
	HgT
	SSC
	PCBs
Sieves	Microplastics

Need all bottles to be half filled to collect minimum req'd vol			Aliquots (min to max number, and req'd volums in ml)
Analyte	Bottle Size	Min. Vol.	5 to 12
HgT	2 L	125 ml	100
SSC	2 L	300 ml	100
PCBs	2.5 L	1 L	200

	Start and End Time (Military Standard Time)	Turbidity Cell(s) & Notes
Aliquot 1		
Aliquot 2		
Aliquot 3		
Aliquot 4		
Aliquot 5		
Aliquot 6		
Aliquot 7		
Aliquot 8		
Aliquot 9		
Aliquot 10		

Notes/Additional Turbidity Samples

Appendix C: Detailed Laboratory Procedures

1. Laboratory Sample Preparation: Water, Sediment, and Fish

At the writing of this SAP, USEPA has not developed a standardized laboratory method for all matrices. NOAA has developed a method for water and sediment (Masura et al. 2015); however, as noted in the body of the Sampling and Analysis Plan, there are some limitations associated with this method (e.g., Dehaut et al. 2016; Lusher et al. 2016). As a result, recent literature methods that have been successfully demonstrated will be used and are cited below.

Water (surface water, stormwater, and wastewater)

Manta trawl, pump system, stormwater, and wastewater samples will be processed and analyzed for microplastic by the University of Toronto Rochman laboratory. Samples will be subjected to digestion to remove labile materials; the digestion will take place at room temperature using 4N KOH solution for up to 14 days. A solution of KOH is preferred as research to date has shown that more aggressive techniques can result in the dissolution of some types of microplastic (Dehaut et al. 2016; Lusher et al. 2016). After digestion, the sample will be filtered through a 10 micron filter and analyzed using Raman spectroscopy. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, chemical composition, and particle type will be reported. Photographs of the particles will also be included in the data package.

Sediment

If sediment samples contain a high amount of organic material, the samples will be subjected to digestion at room temperature using 4N KOH solution for up to 14 days. If minimal amount of organic matter is present, then the digestion step will be skipped and the sediment samples will be dried at 90 °C and added to a sodium polytungstate (SPT) density solution of 1.5 g cm^{-3} using a modified method of Corcoran et al. 2015. The mixture will be magnetically stirred for 2 minutes and then transferred to a glass separation funnel to settle. The non-buoyant material will be drained into a beaker, followed by draining of the buoyant material (low density) into a separate beaker. The low density material will be rinsed thoroughly with distilled water and filtered onto a polycarbonate filter for analysis via Raman spectroscopy. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, chemical composition, and particle type will be reported. Photographs of the particles will also be included in the data package.

Prey Fish

Fish will be thawed and dissected to remove gut and gut contents for digestion, consistent with previously published protocols (Dehaut et al. 2016; Foekema et al. 2013; Corcoran 2015). The guts and contents will be placed in a jar filled with 4N KOH solution. The amount of KOH added will be at least 3 times the volume of biological tissue. The material will be left at room temperature for up to 14 days to

facilitate the digestion. The jars will not be stirred to avoid damage to plastic from hard materials such as rocks, shells, etc. After digestion, the sample is filtered through a 10 micron filter and analyzed using Raman spectroscopy. Spectroscopy will be used to chemically confirm the polymer identity of the microplastic using a reference spectra library. Information on particle size, chemical composition, and particle type will be reported. Photographs of the particles will also be included in the data package.

2. Laboratory Analyses: Microplastic and Nanoplastic

Microplastic samples (20 microns and above)

Samples will be analyzes using the following laboratory protocols by Dr. Chelsea Rochman and collaborators at the University of Toronto.

If a water sample, i.e., not already on a filter, follow steps 1-8. For pump samples that are on a polycarbonate filter (i.e., surface water pump samples, sediment, fish), go to step 9.

1. Wipe down, rinse and set up manifold with 10 micron polycarbonate filter in the clean cabinet.
2. Before each sample is filtered (and in between samples), rinse all glassware and the manifold that will touch plastic. Flush filter with 1 L milliQ water.
3. Filter each water sample through filter paper. It is possible that more than one filter may be needed if the filter clogs. If water is moving more slowly through filter column, change the filter (i.e., don't allow the filter to clog). After all the water in the sample jar has been passed through the filter, the sample bottle will need to be rinsed to assure all particles are removed. Add MilliQ water to the bottle, cap the bottle, shake the bottle vigorously, and then pour the contents over the filter. Repeat this two more times to assure sample bottle is devoid of microplastic particles.
4. Allow clean air (keep tube in bottle and in clean hood) to go through filter for 5 minutes.
5. Do not release pressure valve before taking off lid, as it will make filter stick to top. Immediately remove filter from manifold using clean tweezers and place into a clean glass petri dish and cover with lid; when dry seal it with laboratory tape (all the way around).
6. Run a blank every 10 samples, or at least once per day.

7. Each sample will be analyzed using Raman Spectroscopy. Place dry filter paper under Raman scope. Use Particle Finder software on instrument to find and characterize all particles. Scanning Raman will then analyze the chemistry of each particle. For those whose spectra match synthetic polymers in the polymer library, we will also measure length, width and area. In addition, take a picture of each particle that is determined to be plastic.
8. Place filter back in petri dish and cover with lid, and when dry seal it with laboratory tape (all the way around). Samples should be labeled and saved until project is complete.

Nanoplastic samples (< 1 micron)

Surface water, sediment, and fish samples will be analyzed for nanoparticles by the Duhaime/Banaszak Holl Lab at the University of Michigan. This research group will develop and implement methods to collect and identify nanoplastic debris in aquatic habitats.

The primary analytical approach will rely on the combined application of infrared (IR) spectroscopy (Figure C-1) and atomic force microscopy (AFM). The AFM-IR technique (Anasys nanoIR2) will simultaneously provide information about particle morphology and chemical content for particles ranging from tens of microns to tens of nanometers. Information on particle size, morphology, and chemical composition will be reported. In addition, images of particles will be submitted with the data package. If fields of particles are observed, statistical data will be provided; an example is provided in Merzel et al. 2016. This technology, photothermal infrared spectroscopy (PTIR) or AFM-IR spectroscopy, can be used to directly take IR spectra on a particle-by-particle basis and even map subcontent within a particle. This capability is important to characterize bi-component particles and fibers and polymer blends. The spatial resolution (~10-50 nm) is substantially better than traditional FTIR microscopy (~2000-5000 nm) and is a nice complement to Raman spectroscopy because it is not influenced by the presence of dyes or strongly absorbing fillers. AFM-IR has been successfully applied to spatially map the chemical content of diverse materials, including industrial application for the identification of polymeric and biological materials. The laboratory also has an FTIR microscope and a Raman microscope if complementary methods are required.

Specific collection methods are described in the preceding sections. Briefly, for water we will use the approach of Barrows et al (2016), collecting 1 L grab samples. If sufficient numbers of particles are present for direct deposition, isolation onto filters, or formation of Langmuir-Blodgett layers, this approach will be continued. If insufficient particles are present in 1 L grab samples of surface water, the group will attempt to develop a filtration method similar to that described for microplastic. This may be challenging due to the flow rates possible through micro to nano pores. Sediment samples will be collected into a one-liter pre-cleaned glass bottle. Fish samples will be collected; individual fish will be wrapped in foil and sent to the laboratory.

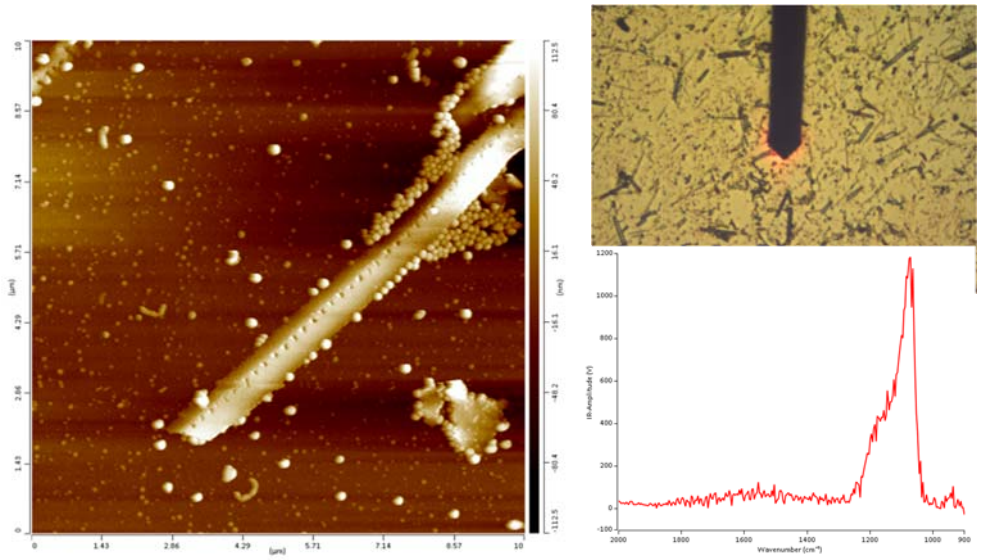
The research group will develop a novel technique for preparation of surface water, sediment, and fish samples for analysis using a nanoIR2 AFM-IR (Anasys Instruments; Santa Barbara, CA). Labile organic matter in the sample will be degraded and removed, putative plastic particles >1 µm will be analyzed through other means, and putative plastic particles <1 µm will be concentrated onto a clean surface (i.e., mica, graphite, MoS_2), Langmuir-Blodgett layers, or onto filters (i.e., anodized aluminum with nanopores) for nanoIR2 scans.

Sediment samples will be subjected to the same isolation protocols described for microplastic. Direct deposition on clean substrates (i.e., mica, graphite, MoS_2 and chemically modified forms thereof) will be employed if sufficient particles numbers are obtained. If not, Langmuir-Blodgett or filtration strategies will be employed. Fish sampling will follow the strategy described for microplastic; however, we will also

employ a final 2-micron particle filter to remove larger particlulates. The filtrate will be analyzed for nanoplastic.

The resulting data will include chemical identification of nanoscale particles and morphological analysis. Not only will these results inform whether nanoplastics are present and their abundances in these habitats, this project will generate a library of IR scans to be made publicly available to leverage community-wide research efforts in the identification of environmental plastic debris.

Figure C-1. Atomic force microscopy-infrared spectroscopy (instrument)



(left) AFM image of diatom and nanospheres collected from Lake Erie water samples. (right top) nanoIR cantilever; AFM-IR adsorption spectra are created by measuring the vibration of the cantilever at different wavelengths of radiation. (bottom right) resulting ATM-IR spectra. (R. Merzel and R. Cable; University of Michigan)

3. References

Corcoran P, Norris T, Ceccanese T, Walzak M, Helm P, and C Marvin. 2015. Hidden Plastic of Lake Ontario, Canada and their Potential Preservation in the Sediment Record. Environmental Pollution. p 17-25.

Dehaut A, Cassone A, Frere L, Hermabessiere L, Himber C, Rinnert E, Riviere G, Lambert C, Soudant P, Huvet A, Guillaume D, Paul-Pont I. 2016. Microplastic in seafood: Benchmark protocol for their extraction and characterization. Environmental Pollution.

Fokema E, Gruijter C, Meriga M, van Franeker J, Murk A and A Koelmans. 2013 Plastic in North Sea Fish. ES&T 47. 8818-8824

Appendix D: Chain of Custody

Chain of custody records will be maintained throughout the course of the sampling effort. For each set of samples being shipped to a laboratory or archive, the subcontractor (CCR) will initiate a COC form and include the original form with the sample shipment, and provide a copy/scan of the form to SFEI data management team at the time of the shipment.

The attached COC form can be used for all samples. The sample matrix (surface water, sediment, prey fish, stormwater, or wastewater) can be specified in the “Matrix” field. For surface water samples, note the type of sample (manta trawl, pump, or 1 L) in the notes field.

Chain of Custody Record

Results to:	Invoice to:	Ship to:
San Francisco Estuary Institute 4111 Central Ave Richmond, CA 94804 Phone: 510-746-7334 Fax: 510-746-7300	San Francisco Estuary Institute 4111 Central Ave Richmond, CA 94804	

Sampled by [Print Name(s)] / Affiliation	Project Name: 2017 Moore Microplastic Study
Sampler(s) Signature(s)	Billing Code: 1111.00/ / 3 / 531.10

Sample ID	Sampled: Date	Sampled: Time	Matrix	Container Type/#	Analyses Requested: Micro (U of Tor) - 500ml	20 Micron (U of T) - Filter	Nano (U of MI) - 1-L	Collection jar (SW / WW)			Notes (Please indicate type of surface water sample: Manta / Pump / 1L/Stormwater/ Wastewater)
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
			Water								
					← Total number of containers						

Relinquished by (Signature) / Affiliation	Date	Time	Received by (Signature) / Affiliation	Date	Time

Shipping Information	Additional Comments
Shipping Date: Courier: Number of Coolers: Cooler Temperature (C):	