Recommended Best Practices for Collecting, Analyzing, and Reporting Microplastics in Environmental Media: Lessons Learned from Comprehensive Monitoring of San Francisco Bay

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Abstract: Microplastics are ubiquitous and persistent contaminants in the ocean and a pervasive and preventable threat to the health of marine ecosystems. Microplastics come in a wide variety of shapes, sizes, and plastic types, each with unique physical and chemical properties and toxicological impacts. Understanding the magnitude of the microplastic problem and determining the highest priorities for mitigation require accurate measures of microplastic occurrence in the environment and identification of likely sources. The field of microplastic pollution is in its infancy, and there are not yet widely accepted standards for sample collection, laboratory analyses, quality assurance/quality control (QA/QC), or reporting of microplastics in environmental samples. Based on a comprehensive assessment of microplastics in San Francisco Bay water, sediment, fish, bivalves, stormwater, and wastewater effluent, we developed recommended best practices for collecting, analyzing, and reporting microplastics in environmental media. We recommend factors to consider in microplastic study design, particularly in regard to site selection and sampling methods. We also highlight the need for standard QA/QC practices such as collection of field and laboratory blanks, use of methods beyond microscopy to identify particle composition, and standardized reporting practices, including suggested vocabulary for particle classification.

Keywords: marine microplastics; oceans; streams; sediments; aquatic ecosystems; best practices; quality assurance/quality control

1. Introduction

Microplastics, tiny pieces of plastic smaller than 5 millimeters, have been recognized as a pervasive and preventable global threat to the health of aquatic ecosystems. Microplastics are ubiquitous contaminants in the ocean [1], and have been observed in every setting examined, even remote locations such as the Arctic [2,3] and deep sea sediment [4,5]. Once introduced into the environment, microplastics become a highly persistent part of aquatic ecosystems [1]. The half-lives of microplastics are not known with certainty due to the broad variety of polymers in use and varying environmental conditions; however, it is widely believed that these particles are resistant to environmental degradation and will persist in the environment long after their release [6–8].
Aquatic organisms at every trophic level are exposed to microplastics [9–11], but the health risks posed are uncertain due to variation in plastic type, shape, and size, as well as species and exposure [12]. Ingested microplastics can impact the biochemical and physiological processes of many different types of animals [11,13–16]. Microplastics can expose organisms to potentially harmful chemicals, especially plastic additives such as flame retardants, plasticizers, or dyes [17,18]. Microplastics and harmful plastic chemical ingredients and additives can also be transferred up food chains [19–23].

Based on the increasing amount of plastic debris accumulating in aquatic habitats [24,25], current policies are inadequate to address the growing and widespread threat of microplastic pollution. While policies addressing single use plastics and microbeads have been gaining traction, few studies have documented or measured the effectiveness of these reduction strategies [26]. Occurrence data to inform policy decisions are essential to understanding and minimizing the impacts of microplastics. Despite their recognized potential for harm and persistence in the environment, the sources and generation of micro-sized pieces of plastics; the pathways by which these tiny particles reach estuarine and marine environments; microplastic transport, distribution, and fate within these ecosystems; and the levels at which they are taken up into the food web are not well understood. Numerous studies are being conducted to begin to characterize this class of emerging, unregulated contaminants and address the many scientific questions and data gaps that have been identified. The scientific understanding generated by these efforts is critical to informing effective solutions for plastic pollution, and would be improved by implementation of common practices to make studies more comparable.

In 2015, a preliminary screening study of microplastics in the San Francisco Bay indicated that levels of contamination might be higher than observed in other large, urbanized water bodies [27]. These findings suggested the need for a comprehensive regional study to characterize microplastics in the Bay, the potential pathways by which they enter the Bay, and the circulation patterns that drive spatial variation within the Bay and deliver microplastics to the ocean. To develop critical baseline data and inform solutions, the San Francisco Estuary Institute and the 5 Gyres Institute, with analytical expertise from the Rochman Lab at the University of Toronto, collaborated to implement a comprehensive regional study of microplastic pollution in a major estuary [28–30]. This three-year project supported multiple sampling and analysis techniques to develop improved knowledge of anthropogenic microparticles, including microplastics, in San Francisco Bay and adjacent National Marine Sanctuaries.

This review presents a discussion of recommended best practices based on this comprehensive assessment of microplastics in San Francisco Bay water, sediment, biota (prey fish and bivalves), urban stormwater runoff, and wastewater effluent, as well as microplastics in surface water in three National Marine Sanctuaries hydrologically linked to the Bay. The San Francisco Bay case study is instructive, as there is an urgent and critical need to develop standard methods and QA/QC practices for measurement of microplastics [31–34]. Standard methods allow comparison among studies to identify areas of concern and trends that can inform policy and management actions. It is imperative to use appropriate QA/QC methods to ensure microplastic measurements reported for environmental samples are accurate and not significantly influenced by background contamination in the field or laboratory. Implementation of QA/QC measures also allows researchers to assess variation in microplastic analyses to determine whether variation observed in the field is statistically significant or merely a reflection of the variation in collection and analysis. Development and implementation of standard methods and QA/QC procedures will allow robust evaluation of management actions to ensure they are being implemented effectively and in areas where they will have the greatest impact.
2. **Microplastics vs. Microparticles**

Microplastics are commonly defined as plastic particles smaller than 5 mm in at least one external dimension [13,35,36]. The lower size limit of microplastics within a sample are operationally defined by the mesh, sieve, or filter pore size used in sample collection and analysis. For example, manta trawl collection of surface water samples typically captures particles greater than 0.355 mm, which corresponds to the mesh size of the net, the collection end of the sampling apparatus, and the sieves used. Some particles smaller than this operational lower size limit may be captured due to aggregation or association with larger materials. In contrast, particles like fibers, which are long and thin, may in some cases escape capture depending on their orientation and movement relative to the net [37–39]. Particles smaller than 0.0001 mm (or 0.1 μm) are generally defined as nanoparticles [36], and have yet to be quantified in a field sampling campaign due to methodological barriers.

The term *microplastics* encompasses a wide variety of plastic materials, each with unique physical and chemical characteristics. The term *plastic* is quite broad and generally refers to any synthetic water-insoluble polymer (typically of petrochemical origin) that can be molded on heating and manipulated into various shapes designed to be maintained during use [13,40]. The California definition of microplastics is more inclusive, and includes non-malleable polymers, as well as some soluble polymers [41]. Common polymers are polyethylene (PE), polypropylene (PP), polystyrene (PS), nylon (polyamide), polyethylene terephthalate (PET, or polyester in the case of fibers), acrylic (polyacrylonitrile and related polymers), and polyvinyl chloride (PVC), among others [42,43]. Rubber, whether natural (isoprene) or synthetic (e.g., styrene-butadiene), may also be considered a plastic, and is frequently included in the field of microplastics [44]. Many plastics contain chemical additives to impart desirable characteristics, including flame retardants, plasticizers, and dyes.

Microplastics are frequently described as primary or secondary, to distinguish between the general types of sources from which they originate. Primary microplastics are designed and manufactured to be small for a variety of uses, including pellets for plastic production (e.g., “nurdles”), abrasive blasting, paints and adhesives, agricultural applications, and for use in personal care products [6]. Primary microplastics may be released into the environment as a consequence of spills, use of products that intentionally release them during maintenance and use [42]. In contrast, secondary microplastics originate from the degradation of larger plastic items, regardless of when this breakdown occurs [8]. Under these definitions, cosmetic beads used in bath products, particles used for sandblasting, and preproduction resin pellets are examples of primary microplastics, while tire road wear particles, fibers from textiles, and particles formed from the breakdown of plastic litter are examples of secondary microplastics.

The first studies in the field of microplastics relied on identification using only visual techniques, such as microscopy. Studies using additional, advanced laboratory techniques demonstrated that a portion of these visually identified particles were erroneously characterized as plastics, an issue that becomes more likely with decreasing particle size [43]. Therefore, the use of spectroscopy or other chemical identification methods is essential for accurately characterizing microplastic pollution. In more recent studies, laboratory analysis of microplastics typically involves microscopy as a first step to identify particles that visually appear to be plastic, followed by a confirmation step to establish that the particle is, in fact, plastic, and to determine what type.

To provide transparency around the level of certainty regarding particle composition, we recommend carefully distinguishing between suspected anthropogenic microparticles (often shortened to just microparticles), a term we would define as small particles (less than 5 mm) that are visually identified as potentially plastic, and microplastics, which have been confirmed to be plastic through spectroscopy or
other chemical techniques. This is an important and necessary distinction because some particles that visually appear to be plastic are anthropogenic, but made of other materials (e.g., glass, metal, cotton), and not all potentially plastic particles can be confirmed as plastic, either due to resource constraints or analytical challenges.

3. San Francisco Bay Microplastics Monitoring

3.1. Study Setting: San Francisco Bay and Adjacent National Marine Sanctuaries

San Francisco Bay is the largest estuary on the west coast of the Americas. The climate of wet winters and dry summers means that inputs of freshwater and associated chemical and biological constituents are highly seasonal. Seawater enters through the narrow deep channel at the Golden Gate, and freshwater is delivered primarily by the Sacramento and San Joaquin rivers. About 90% of the freshwater flow into the Bay comes from these rivers, which drain over 40% of the state, including a major inland agricultural area with several large cities such as Sacramento, Fresno, and Bakersfield. Much smaller contributions of freshwater are delivered via small local tributaries, particularly during the wet season. Active flushing of the northern and central portions of the Bay results in generally cleaner water quality than the southern portion of the Bay, which only receives about 10% of the freshwater flow.

The Bay and three National Marine Sanctuaries outside the Golden Gate (Greater Farallones, Cordell Bank, and Monterey Bay) are a unique ecosystem and an important habitat for a wide range of species. It provides spawning, nurturing, and hatching grounds, as well as a migration route stopover for anadromous and marine species and birds on the Pacific Flyway. The Bay Area is also highly urbanized, home to over seven million people, and supports diverse industries spanning such sectors as electronics and semiconductors, metalworking, apparel, food and beverage, and lifestyle products [45]. As a result, approximately 1.2 billion cubic meters of treated wastewater per year is discharged from 42 wastewater treatment plants [46]. Small tributaries surrounding the Bay contribute flows primarily driven by stormwater inputs in the wet season. Flows are highly variable but are estimated to be about 12 billion cubic meters per year [47], compared to 25 billion cubic meters per year from the Sacramento and San Joaquin Rivers.

San Francisco Bay has long been monitored for many regulated and unregulated contaminants of interest due to community support for regional monitoring [48]. It is also a good system to study urban influences on water quality because of the unique combination of attributes, including its high population density and diversity of industries, ecological importance, and contribution of stormwater runoff and wastewater. Because of its hydrodynamics, the Bay also acts as a long-term trap for persistent contaminants, with recovery taking decades or longer when contamination is extensive (e.g., mercury and polychlorinated biphenyls) [49]. Given this combination of characteristics in the Bay, much can be learned about the scope of contamination and potential impacts of accumulative anthropogenic contaminants such as microplastics.

3.2. Study Objectives and Rationale

The overall objective for the recent comprehensive San Francisco Bay microplastics monitoring was to improve understanding of microplastics in the Bay, with a focus on source identification, estimation of relative loads, and occurrence in the Bay and sanctuaries to inform policy and management actions. An additional goal was to advance the field by implementing a rigorous study design and developing and refining field and analytical methods and quality assurance practices that could be applied broadly. This
project supported multiple sampling and analysis techniques to develop improved knowledge about and characterization of microparticles and microplastics in San Francisco Bay and adjacent National Marine Sanctuaries, with specific objectives and corresponding scientific needs listed in Table 1. Below we outline the study design elements that we considered and how we used these objectives to drive our decisions. The objective of this paper is to report lessons learned from our quantification of microplastics in various matrices in San Francisco Bay, so that others may learn from our successes and our mistakes.

4. Lessons Learned

4.1. Sample Site Selection and Field Sampling

4.1.1. General Considerations for Sampling Design

4.1.1.1. SITE SELECTION

In theory, many of the key fundamentals of sampling design for microplastics are the same as for other environmental contaminants (e.g., choosing a targeted vs. a probabilistic design, developing the design to answer specific statistical questions). The best way to get truly representative sampling is through probabilistic sampling with an adequate sample size, while targeted sampling is generally more powerful for trend analysis. However, regardless of the sampling design, the exorbitant current cost of microplastic laboratory analyses (time and labor, leading to monetary costs) is a major obstacle to adequate sampling for statistically robust data on environmental concentrations. Study design should therefore include careful consideration of how to make the most of a small number of samples. This cost-benefit analysis is even more important when considering the high number of recommended quality assurance samples, and further emphasizes the need for automated methods (discussed below).

A focus on trend monitoring necessitates a design with carefully located sampling sites, and controlling for season, tidal stage, current, and other spatial and temporal factors expected to influence concentrations. For our study, one of the primary objectives was to establish a baseline dataset for future monitoring. This dataset will provide a starting point for future sampling to detect differences among sites and over time.

We selected sampling sites based on geographical coverage, proximity to potential pathways and undeveloped areas, potential to capture species of interest, and seasonality [50]. Additional important factors were needs for model development [51], as well as ease of access and our ability to leverage other sampling efforts for this project. As a result, sites in the National Marine Sanctuaries were chosen to represent conditions in surface waters above the continental shelf that extends several kilometers from shore and above the continental slope that drops steeply into the deep ocean. Care was also taken to retain sampling locations both within and beyond the influence of the San Francisco Bay freshwater outflow. In the Bay, sites were chosen to range from the deeper open Bay to the shallow margins. This information was important input for model development, and allowed us to characterize conditions across the sanctuaries and Bay.

The geography, hydrology and hydrodynamics, and bathymetry of the water body, as well as potential sources (e.g., outfall or discharge points) should be considered as part of the study design. In order to adequately capture spatial variation in contaminants (for microplastics, this includes concentrations and material type) caused by these factors, larger study areas can be subdivided into regions. For example, the Bay has been divided into five subembayments for monitoring based on geography, hydrodynamics, best professional judgement, and management needs [52]. If cross-matrix interactions and dependencies are being examined, sites should be located and distributed to
complement the sampling plans for other matrices to the extent possible. We preferentially chose many sites along the margins of the Bay due to their habitat importance and proximity to the major pathways we were investigating (stormwater outfalls, as well as some wastewater discharges).

Desirable sites may be in areas that require additional planning. For example, surface water sites may be in or near restricted zones around airports or in logistically challenging areas, such as active shipping lanes or ferry transit corridors. Shallow sites require careful coordination with tidal cycles to avoid vessel stranding. Stormwater sites may also require special permits or approval to sample, or present access challenges due to the site location (e.g., busy highway bridges). Wastewater sample collection requires approval and support from the wastewater treatment facility leadership and staff. Site specific sampling equipment may need to be developed, given the wide range of conditions at treatment facilities and stormwater sites. Scientific collection permits, access permits, and approval to sample parks, protected areas, or other locations can take several months to obtain.

Where possible and appropriate for addressing the study objectives, inclusion of a less urban reference site can provide valuable context for understanding the degree of contamination in the primary study area. A good reference site for urban microplastic monitoring is a location that is similar to the study site (i.e., similar climate, geography, geology, and hydrology) but is rural and undeveloped. The surrounding watershed should be largely in pristine condition with limited impervious surfaces and little transportation infrastructure. Sites that have wastewater and stormwater outfalls should be avoided. We selected Tomales Bay as our reference site, which is located 45 km north of San Francisco Bay and is bordered on one side by a national park and on the other side by a low population density, rural area. Past examination of other contaminants there have shown lower concentrations in biota relative to the more urbanized Bay (e.g., [53]). A reference site is particularly important for studying biota so differences in contamination due to relevant site conditions (e.g., urbanization) can be identified.

Figure 1 shows the study area, sites, and matrices sampled in San Francisco Bay and in Tomales Bay, the reference site.

4.1.1.2. FIELD SAMPLING

The selection of sample collection methods is critical and will dramatically influence the study results [37,54–57]. With this in mind, it is imperative to think about the study questions and how the data will be used to answer these questions. There are four key questions regarding collection methods to consider:

- **What is the lowest size fraction of interest and why?** In general, the smaller the particle size of interest, the more particles will be collected for a given volume of sample [38]. For example, microparticle concentrations reported in wastewater in the literature range four orders of magnitude from the largest size fractions to the smallest [28,58–61]. Smaller particles can be harder to trace to sources, so if the goal of the study is to investigate sources, it may not be necessary to use less than a 125 μm screen sieve.

- **How will the data be used relative to other datasets?** If the goal is to compare among matrices (e.g., to assess the relative importance of loads or food web accumulation), it is critical to standardize the mesh size of sieves across matrices to facilitate comparisons via consistent operational size categories. If the goal is to compare to past measurements or those reported in the literature, methods consistent with prior studies or literature studies of interest should be used.

- **What type of particle morphology is of interest and why?** Fibers are the most challenging morphology to collect and analyze due to their potential for sample contamination (see below), as well as their elongated shape and thin diameter. Depending on the orientation of the fiber, it may not be caught by
a net or sieve, which can result in significant undercounts [37,38,57]. This can make comparisons among results from different sampling procedures challenging. If the goal of the study design is to accurately capture and quantitatively assess fibers, it is more appropriate to use bulk grab samples rather than net sampling methods. Filters with pore sizes small enough to prevent fiber passage should also be used.

- How much volume to sample? Large sample volumes are often desirable because they are less affected by the heterogeneity of the matrix, and therefore are more likely to be representative. In addition, a larger volume may be necessary to overcome background levels of contamination. However, given the labor intensity of sample extraction and analysis, the smallest sample size should be collected; otherwise subsampling may be necessary. We suggest piloting methods before employing them, as a small pilot study can help to calibrate and refine methods by determining expected microplastic concentrations. Replicate samples (approximately same time/place) can be especially valuable to assess variability in observed concentrations.

4.1.2. Matrix-Specific Considerations for Sampling Design

The recent San Francisco Bay microplastics study was one of the first to conduct a comprehensive and integrated assessment of microplastic concentrations and characteristics in an urban estuary. Microplastics throughout the Bay and surrounding ocean were assessed in five different environmental matrices: surface water, sediment, biota (prey fish and bivalves), stormwater, and treated wastewater effluent. Many of these matrices can be sampled using a variety of methods, each of which has pros and cons. In general, we tried to consider factors that could influence microplastic inputs and movement through each matrix, and chose methods based on the data they could provide. Our goal was to collect samples that were as representative as possible of ambient conditions and microplastic loads entering the Bay. Each matrix presented its own unique challenges for sampling design and implementation. Here, we present lessons learned from each matrix sampling effort.

4.1.2.1. SURFACE WATER

Hydrodynamics are an important consideration for surface water site selection. For example, the Central Bay region of San Francisco Bay is a convergence area of water from the north and south embayments and receives tidal inflow from the open ocean, leading to strong gradients in water properties. These attributes complicate particle transport through the region and motivated additional sample sites to constrain predictive models [51]. Another consideration is that tides, currents, and wind can concentrate buoyant and semi-buoyant material in surface water features such as tidal fronts, windrows, and eddies [62]. We had at least one instance of sampling in which the manta trawl passed through a prominent tidal front halfway through sample collection. Vegetation, woody debris, trash, and plastic fragments were observed floating at the surface and were captured in the sample. This sample had the highest microparticle abundance recorded within the study, and could be indicative of the microparticle levels found in these biologically important fronts within the Bay and the nearshore marine sanctuaries. If a feature like this is unexpectedly encountered (and noticed), and if resources allow, it would be ideal to have samples within the feature, as well as nearby but outside of the feature, to assess its potential impact on study observations.

Water samples from oceans and lakes are typically collected within one meter of the water surface, rather than at deeper depths or depth-integrated; relatively few studies have explored the vertical profile of microparticles with depth or sampled significantly beneath the surface water [63,64]. Surface water samples are most frequently collected using manta trawls or neuston nets, which are designed to skim the
surface, although bulk water pump systems or grab samples have also been employed [37,54,65,66]. Pump and grab samples require far less effort and sample smaller volumes; however, due to their smaller volume, they are more easily influenced by heterogeneity and so may be less representative of the surrounding surface conditions, especially for larger microparticles.

Comparison between neuston nets and grab samples showed that grab sampling collected over three orders of magnitude more microplastics per volume of water, as well as a larger size range than sampling with a neuston net [37]. Grab samples are able to characterize all morphologies, can capture smaller particles, and can be used in environments where nets are impractical, but the small volume of water sampled may result in high variability among samples [67]. We explored using a one-liter grab; however, based on our results, a larger grab sample volume would be recommended to overcome background contamination [57]. Others have recommended that at least two to four liters be used [34]; we recommend piloting methods to determine relative sample and background concentrations.

Potential seasonal differences in surface water concentrations should also be considered. We collected samples during dry and wet seasons, and observed statistically significantly higher concentrations following wet weather events (defined below). Studies in other locations have also observed significant connections between rainfall and microplastic abundance in surface waters [e.g., 68,69].

4.1.2.2. SEDIMENT

It is important to consider whether sediment sites are located in areas that are depositional or erosional, and if there are unique attributes that could impact microplastic levels locally, such as known trash hot spots or areas of active dredging or deposition of dredged material. We chose sediment sampling sites to characterize ambient conditions and the influence of potential pathways for microplastic transport; therefore, most were located in the nearshore Bay margins and none were influenced by dredging. These areas are nearest to and likely most affected by stormwater runoff from urban creeks and shallow wastewater discharges, and are frequently depositional rather than erosional. We co-located sediment and fish sampling sites to facilitate the study of bioavailability.

Sediment samples can be collected using a variety of devices: a sediment grab device (such as a Van Veen, Ekman, or Ponar grab), a bed sediment trap, or a coring device [66,70]. The depth of the sediment to be sampled is an important consideration, with shallow samples more likely to represent more recent conditions. In contrast, sediment cores can give an indication of trends over time, provided the area has remained depositional over time and the sediment has not been significantly disturbed. The European Union Marine Strategy Framework Directive recommends that surface sediment samples be collected from the top 5 cm of sediment, because this layer is affected by seasonal erosion and deposition and thus likely to be most representative of recent conditions, and because that is what most studies have done [71]. This recommendation may also allow monitoring to best account for biological activity within the sediment, and is consistent with most ecological risk assessment bulk sediment screening procedures. We used a stainless steel scoop to sample the top 5 cm of sediment in a Van Veen grab, taking care to avoid the sides of the grab, and deposited the sample directly into a clean glass jar. In the Bay, the surface (top 5 cm) subtidal sediment layer primarily includes sediment from the past few decades, with extensive but heterogeneous mixing in many areas due to bioturbation and abiotic processes [72,73]. Estimating the age of surface sediment is difficult due to mixing and transport of sediment from adjoining margin areas and Bay segments.
4.1.2.3. BIOTA

Biota sampling locations are predicated on the targeted species and their ecological niches. We chose to monitor two species of prey fish, anchovy (*Engraulis mordax*) and topsmelt (*Atherinops affinis*), and two bivalve species, Asian clams (*Corbicula fluminea*) and California mussels (*Mytilus californianus*), to assess microplastics entering the Bay food web. Prey fish and bivalves can serve as indicators of the bioavailability of microplastics, as they are an important food source for piscivorous fish, birds, and marine mammals. Bivalves are also a potential route for human exposure to microplastics in some areas of the Bay; while bivalves are not commercially harvested, they are recreational/subsistence harvested.

The feeding habits of the chosen species may influence the design of accompanying water and sediment sampling. Benthic feeders may more readily ingest microplastics in sediment as they forage, while pelagic feeding species are less likely to encounter this exposure route. Concentrations of contaminants in sediment may be correlated with concentrations in benthic feeding fish, as has been demonstrated for polychlorinated biphenyls in Bay prey fish [74]. We observed fibers to be the majority of particles in fish (86%) and bivalves (98%), indicating the importance of assessing fibers in all matrices when evaluating food web uptake. We chose prey fish species with different foraging habits to explore whether this would also be true for microplastics, and observed higher non-fiber particle abundances in benthic-feeding topsmelt than in pelagic-feeding anchovies.

The home range of the species is also important; organisms with high site fidelity are better for comparing to other environmental matrices, as co-located sampling will more accurately represent exposure. Another reason we chose prey fish is that they exhibit high site fidelity relative to other fish species, allowing for potential identification of areas of higher contamination and concern. Bivalves are stationary and can be transplanted from areas of low contamination to areas of high contamination to directly assess uptake [30]. Mesocosm studies can also help assess contaminant spatio-temporal variation within an aquatic system, and may be useful to apply to microplastics monitoring efforts.

A variety of techniques can be used to sample aquatic organisms; the selection of the method is a function of the targeted species and the habitat in which they reside. It is probably a good practice to target individuals of standardized weight and length to facilitate comparisons among individuals and locations and to reduce variation due to external factors such as age and consumption rates, although we did not see any correlation between size and particle count. Depending on the target species and study questions, it may also be important to collect biometric data to confirm species identity and/or to assess individual fitness. It is important to have flexibility in the study design (i.e., backup sampling sites) in the event that no target organisms are present at the original target site. Care should be taken that collection and transport of samples is as clean as possible, as representative field blanks may not be possible. If the study design calls for dissection and assessment of individual organs, we recommend collecting the whole organism in the field and doing all sample processing in a clean laboratory.

Due to the wide variation in biological samples, it is important to collect sufficient sample sizes for each site for statistical analyses. A sample size of at least 50 individuals per research unit (species, food web, ecoregion, feeding type, etc.) has been recommended, but this number is somewhat arbitrary [32]. If ingestion incidence is low and thus more variable between individuals, larger sample sizes will be needed to give reliable results, whereas smaller sample sizes may be sufficient for populations with a high incidence of microplastic ingestion. We collected ten of each fish species at six sites in the Bay and analyzed them individually. While this sample size is near the minimum number for viable statistical analyses, we were able to detect some differences between species for this level of cost and effort [28]. Analyzing samples of individuals from each site provided us with more granular data by which we could...
assess statistical power to detect differences among sites, as well as providing more information about individual differences. For example, particle counts distribution skewed to the right, with many fish containing fewer particles than the average, and a few individuals having high particle counts. In contrast, we collected composites of 4–10 bivalves per site [30]. Composite sampling results in fewer analyses and provides a more integrative measure; however, it also decreased our ability to detect differences between sites and species, and would have required many more samples to obtain a sufficient number of composites to assess statistical differences.

4.1.2.4. STREAMS AND STORMWATER RUNOFF

To develop models, it is important to understand relative loads discharged by pollution pathways like stormwater runoff. Small tributary sampling design should include a sufficient number of watersheds and span the range of expected variation to begin to characterize the pathway as a whole. To assess potential sources, tributary site selection should account for the land-use characteristics in the watersheds of interest. Including watersheds with a range of land-use profiles indicated by varying percentages of land area classified as agricultural or open space, commercial, industrial, residential, or transportation helped us assess whether certain land uses (that could potentially be used to tie microplastics to their sources) were associated with the generation of higher microplastics loads. We sampled 12 watersheds, which together comprise 11% of the total small tributary watershed area draining into the Bay, with total urban area within the watersheds ranging from 9%–98%.

It is also important to consider the influence of seasonality and storm-related transport on loads. Research suggests significant increases in microplastic loading during wet weather events [75]. With a Mediterranean climate, Bay Area rainfall largely occurs November–April. Because 95% of the flow in Bay Area small tributaries is the direct result of rainfall [76], and dry weather sample collection may be less important for load calculations, we focused our tributary sampling during rainfall events that occur during the winter and spring. In the literature, much of the focus is on dry weather sampling [77,78], but our results indicate stormwater delivers relatively large loads of microplastics during wet weather [28].

We recommend establishing a storm threshold level for sampling a wet weather event prior to starting a study. We chose to collect samples during storms that were predicted to have more than 1.3 cm of rain within six hours [50,79]. Based on prior studies of legacy contaminants in Bay Area watersheds, these conditions are sufficiently intense to mobilize small particles from the watershed [80]; storms forecasted for shorter duration and smaller magnitude often result in little runoff. This threshold will likely be site-specific; for the Los Angeles Basin a criteria of 0.6 cm over 24 hours was used to define a wet weather event [73]. For areas that receive regular rainfall, the time between wet weather events (e.g., number of days without rain) may also need to be specified. In the Bay Area, rainfall is so sporadic that this consideration was unnecessary.

We recommend being on-site at the beginning of the storm to sample the start of storm-related flow, as contaminants can be mobilized off the landscape by the first flush [81], particularly in places like California where there are long periods of dry weather. If possible, we also recommend choosing tributaries that have streamflow gauges so flow-weighted samples can be collected and loads more accurately estimated. Where this is not possible, a time-weighted composite sample can be collected.

Studies of microplastics in streams and stormwater have been scarce, and most have not adequately characterized the microplastics that may be present throughout the water column. We recommend collecting depth-integrated samples for accurate loads estimation. Samples have been collected using manta nets, hand nets, pump systems, or discrete bulk water grabs [75,82]. Using a net or pump system measures the average over time, whereas a grab sample gives a snapshot of a moment during the storm.
Although the turbulent nature of storm-driven stream flows may cause considerable mixing within the water column, denser materials may still be under-represented by surface samples, which is why we chose to depth-integrate. It is possible that depth-integrated sampling could underestimate the lighter weight microplastics floating on the surface, which can comprise a disproportionate amount of the overall composition of microplastics in some settings [83]. We sampled using an alternative method more likely to capture microplastics at the surface of the stream (using an 11 L stainless steel pail) at a single site in our study due to logistical constraints, and our results suggested that this method might underestimate microparticles overall. Horizontal integration may also be important, and recent efforts to determine best sampling practices by estimating particle location with streams based on fluid dynamics recommend both depth and width integration [84].

4.1.2.5. WASTEWATER

Wastewater treatment facilities process influent using a variety of treatment methods. Understanding the potential influence of these different methods requires sampling at facilities that employ different processes. To provide robust estimates of overall loads to the water body, the major wastewater discharges must be captured. We selected eight municipal wastewater treatment facilities, including many of the largest dischargers in the Bay Area. These eight facilities represent approximately 70% of the total flow of effluent to San Francisco Bay, or approximately 887 million cubic meters of treated wastewater per year [46]. Four of our selected facilities employed secondary treatment (biological treatment), and four employed additional tertiary treatment that included dual media filtration as a finishing step. One of the secondary facilities treated a combined flow of stormwater and wastewater influent, while all other facilities treated wastewater only.

As with surface water and small tributary sampling, it is important to consider the influence of seasonality. If the sewer system is a combined system, there can be an increase in microparticles observed as a result of stormwater runoff during the wet season, particularly particles related to trash [85]. Effluent from the one combined sewer facility in our study had concentrations of fragments approximately four times the average from the other facilities, even without sampling during a storm. Conversely, wet weather may increase the volume of water coming into wastewater treatment facilities due to infiltration of water into pipes, and thus may decrease concentrations of microparticles observed. We tried to sample only during dry weather to minimize seasonal variability, and because combined stormwater and wastewater treatment is rare in California. We also sampled Tuesday through Friday to facilitate comparison among sites and to provide a more consistent sample, as single facility variation in effluent concentrations on weekends relative to weekdays is well documented for many contaminants [86].

Wastewater effluent samples can be collected as bulk water grabs or grab composites [60], or wastewater can be filtered on-site to provide a composite sample that is more representative of a longer period of time and a larger volume of water [85,87]. We filtered wastewater effluent on-site for 24 hours to limit variability due to variation in flow and composition over a day, rather than over two hours as was conducted in our 2016 pilot study [27]. We observed that some of the secondary treatment facilities had significant algal growth (particularly when sampling dechlorinated effluent), which sometimes clogged sieves over the 24-hour sampling period. We solved this issue by sampling a lower volume by reducing the flow rate at some facilities, and by changing sieves partway through sampling at others. Frequent use and clogging of sieves may cause their performance to degrade over time; it may be important to verify sieve performance before sampling [88].
To capture microbeads, a 125 µm or smaller sieve should be used, as this sieve size has been found to be particularly successful for trapping microparticles used as abrasives in personal care products [89,90]. In a survey of facial cleansers available in the Bay Area, the average diameter of microbeads was 264 µm [91]. Smaller sieve sizes may be needed to adequately characterize microfibers. If calculation of loads is important, collection of flow-weighted and depth-integrated samples could also be considered. We tried to avoid sampling from a quiescent take and instead sample where water should be well-mixed, but were constrained by what was feasible at each facility. Non flow-weighted samples may be biased due to the constant flow from the sampling port. Not depth-integrating across the sampling tank could also create additional bias, although to our knowledge, this has not been tested.

4.1.3. Quality Assurance and Quality Control (QA/QC) in the Field

There is an urgent and critical need to develop standard methods and quality assurance and quality control (QA/QC) practices in the field of microplastics. Relatively few studies have incorporated rigorous study designs, particularly QA/QC measures that are routine in more established fields of trace chemical contaminant monitoring [6–8,56]. Implementation of QA/QC measures allows researchers to assess variation in microplastic analyses to determine whether the differences observed in the field are statistically significant or merely a reflection of the variation in collection and analysis.

It is imperative to use appropriate QA/QC methods to ensure microplastic measurements reported for environmental samples are accurate and not artifacts of background contamination in the field or laboratory. Field QA/QC samples should be collected and analyzed to evaluate the efficacy of methods and to ensure accurate quantification of microplastics [32,33,43,57,60,66,92]. Field QA/QC measures include field blanks, which provide a measurement of procedural and background contamination during sampling, and field duplicates, which are a standard practice for environmental monitoring of chemical contaminants and provide a measure of variability in sample collection and analyses. More replicates per site could provide more statistically robust data, but collecting replicates must be balanced with time and resource costs. Both field blanks and duplicates should be collected in the same manner as the samples. For trace environmental organic and metal contaminants monitored within the Bay via the Regional Monitoring Program for Water Quality in San Francisco Bay, it is standard practice to collect one field blank and one duplicate every 20 samples [93]; however, in this study, due to our concern about external contamination of microplastics, we collected these QA/QC samples at a greater frequency. Blanks are often highly variable and can have high levels of microparticles relative to field samples, especially microfibers. Until sources of external microparticle contamination are better understood, we recommend collecting a field blank during each sampling event, or at least one field blank and duplicate for every ten samples, and possibly more if the study design is focused on microfibers.

Field blanks should be true field blanks, in which every step of the collection procedure is duplicated as closely as possible. Simplified field blanks in which only portions of the process are evaluated do not provide sufficient information to assess the magnitude of blank contamination. For example, for surface water sampling with a manta trawl, it is important that the blank water is flushed through the manta net into the cod end and onto the sieves and then processed onboard in the same manner as the field samples; although it will, by necessity, represent a smaller flush volume. Only pouring the blank water through sieves does not provide a representative indication of the potential for field contamination. Filtered water should be used for blanks so as not to introduce contamination [94]. In addition, a rinse water sample and a bottle blank should be collected to assess the cleanliness of the sample containers.
Field duplicates provide an indicator of variability in field measurements. Any given sample represents a discrete time and place, but because sampled environments are not static and homogeneous, field duplicates can help characterize the degree of variability that is likely to exist for the sample population. Depending on the matrix and sampling method, the collection difficulty and degree of similarity between field duplicates may vary (e.g., for sequential versus simultaneous collections), and thus the acceptable variability between duplicates may also differ. For example, the field duplicates for wastewater effluent in the Bay were collected by dividing the flow from a sample port using a Y-splitter pipe connection, enabling the simultaneous collection of two sample sieve sets in parallel. Stormwater field duplicates were conducted sequentially across the hydrograph, with the primary sample receiving the first aliquot, followed by the duplicate. For both of these methods we expected and observed excellent agreement: less than 30% relative percent difference (RPD) for total microparticles. Inspection of the morphology of the particles in paired field duplicate samples indicated that the majority of variation in wastewater was due to fibers, which was likely a consequence of their orientation and long, narrow shape affecting whether they were captured. In contrast, the majority of variation in stormwater samples was due to fragments, indicating the high heterogeneity of this matrix.

In contrast, collection of a field duplicate for surface water manta trawls was far more challenging because two trawls could not be deployed simultaneously without running the risk of entangling the towlines around the boat propeller. Furthermore, it can be difficult to maintain similar conditions for duplicating sequential trawls (e.g., avoiding the presence of foam lines or convergence zones). For surface water manta trawl sampling in the Bay, the primary sample was collected first (a 30-minute trawl), and then the vessel returned approximately 45 minutes later to the same latitude and longitude of the prior starting point and commenced the second trawl with a similar heading and speed. The RPD for these surface water duplicates varied between 2 to 105%, with open ocean waters having lower RPDs than Bay waters. Although these trawl duplicates were separated in space and time at much smaller scales than individual sites within the overall sampling effort, the variability in the field replicates suggests that large sample counts or scales of integration may be necessary to find statistically significant differences.

4.2. Laboratory Analysis

Methods for characterizing microplastic contamination are rapidly evolving. Microplastics are an unusual and complicated class of analytes; unlike typical chemical contaminants, microplastics vary in size, shape, material type, and additive chemical mixture, and thus require different styles of sample extraction, analysis, and reporting. Analysis of microplastics in environmental samples also requires an experienced laboratory due to the prevalence of background contamination sources and the need for chemical identification that often requires the use of multiple techniques and instruments. Analysis methods have evolved rapidly over the past decade; many older publications only visually identified likely plastic particles (what we term microparticles), while today, the standard has shifted to include material identification methods using spectroscopy, allowing a more accurate identification of microplastics. A full discussion of the available analytical techniques and their pros and cons is outside the scope of this work; others have reviewed this topic in detail [57,95,96]. Few interlaboratory comparisons have been performed to assess method and interlaboratory variability [97].
4.2.1. Quantification of Microparticles and Microplastics

To be enumerated and characterized, suspected anthropogenic microparticles must first be separated from the surrounding environmental matrix. Numerous methods have been developed for separating microparticles from environmental matrices, but there are as of yet no standardized methods and few studies of method efficacy. In general, samples are reduced in volume by sieving; separated from other materials via filtration and/or density separation using a salt solution; and organic matrix matter digested using oxidizing, acidic, alkaline, or enzymatic methods [66]. Methods must be matrix-specific, and there is not yet a clear consensus on the most effective methods for any of these steps for any matrix. It is worth noting that density separation may not successfully separate tire and road wear particles (TRWP). We used a calcium chloride solution of 1.4 g/cm³ density, which is lighter than what has been suggested for TRWP. Although tire rubber and tread particles can have densities[98,99] as low as 1.1 or 1.2 g/cm³, previous reports[100] show the density of TRWP can reach as high as 2.2 g/cm³, indicating our density separation methods may have underestimated the amount of black rubbery fragments in our stormwater and sediment samples.

Although one of the most complex matrices, methods for extracting microparticles from biota have had the most movement towards standardization. For fish samples, it is generally agreed that a digestion step must be included to dissolve organic matter in the sample; simply rinsing the digestive tract does not extract particles entrenched in the tissue. A commonly accepted method is using a 10–20% potassium hydroxide solution or enzymatic methods to digest small aquatic organisms [32]. Heating or drying samples at high temperatures for long incubation periods should be avoided to avoid degradation of plastic particles [32]. Methods for other, larger organisms are still in development, as it is often ideal to sample plastic ingestion without sacrificing the animal (e.g., by making birds regurgitate their stomach contents) [101,102].

For non-chemical separation, standard sieve sizes and fractionation should be used for grouping particles in different operational size categories to facilitate comparisons. In this study, we initially used different size fractions for some matrices, but as we proceeded, we realized how important size fractionation was and sought to standardize size fractions across samples. We also established microparticle size limits for our current analytical methods and instrumentation. For example, in this study our lower size limit was 45 µm, which was based on the smallest size we were comfortable handling with tweezers to prepare them for spectroscopy. This learning process resulted in fractionation that was not always consistently conducted, making comparisons between and among matrices challenging in some cases.

Following extraction, the next step is visual identification of microparticles (i.e., potential microplastics). This process is generally conducted manually and is very labor intensive. As a result, this step can be subjective and influenced by analyst experience and fatigue, which can result in overestimating or underestimating the microparticles present [66]. Even with well-trained staff, it is likely that there will be errors. It is very labor intensive to pick microparticles from contaminated samples, and it can be cost prohibitive and logistically infeasible to analyze all samples or all particles within each sample. Subsampling is often necessary, and current subsampling methods may not always be representative [103]. For these reasons, development of subsampling strategies and new automated techniques that reduce the analytical burden to individual researchers and improve and standardize detection and quantification would be a key contribution to the field. Methods for automated Fourier-transform infrared (FTIR)/Raman mapping or particle tracking with simultaneous Raman spectroscopy
have begun to be explored [104–106], but they require expensive instruments and do not yet work well for samples containing high numbers of particles.

To accurately characterize microplastics in the environment, it is critical to confirm the composition of collected microparticles with methods beyond visual inspection, such as by using selective fluorescent staining or spectroscopic polymer identification. Without material composition information, microparticles of other origins can be erroneously characterized as microplastics, an issue that becomes more likely with decreasing particle size and increasing sample heterogeneity [43,107]. For example, dyed naturally derived microfibers may be lumped in with plastic microfibers, artificially inflating the apparent microplastics in a sample [87]. In our samples, between 11 and 52% of fibers were dyed natural materials.

However, classification of environmental microparticles based on spectroscopy is challenging. Different techniques are more suited to different sizes, morphologies, and materials, requiring laboratories to invest in multiple pieces of specialized equipment. In general, we have found that FTIR spectroscopy methods are best used on larger particles (greater than 250 µm), while Raman spectroscopy is useful for smaller particles. Micro-FTIR techniques can allow accurate analysis of smaller particles. However, Raman does not work well for microplastics that are dyed, particularly those dyed with dark colors because the dye signal can mask the polymeric signal, and dark colors can also lead to fluorescence or burning. In this case, pyrolysis gas chromatography-mass spectrometry may be used to confirm the composition of these particles. There is also currently a dearth of appropriate spectral reference libraries, although spectral reference projects such as OpenSpecy.org are aiming to fill this gap. Weathered plastics have different spectra than corresponding virgin plastic, necessitating the development of spectral libraries specific to microplastics, such as the open access library developed as part of this project [108].

4.2.2. QA/QC in the Laboratory

Similar to field sampling, it is imperative that laboratory QA/QC measures are implemented to evaluate the efficacy of the methods and to ensure accurate quantification of microplastics. These measures include preparing and analyzing laboratory blanks, analyzing reference standards where available, and assessing recovery via matrix spikes, as well as maintaining a meticulously clean laboratory to reduce the introduction of external sources of microplastics.

Laboratory blanks are necessary to quantify and understand the sources of background contaminants in the laboratory, and should be included each time samples are processed and analyzed. As with field blanks, laboratory blanks should duplicate every step of sample processing and analysis as closely as possible. Simplified blanks in which only portions of the process are evaluated do not provide sufficient information to assess the true magnitude of background contamination.

Prior to commencing analyses of field samples, laboratory extraction methods should be assessed. This may be done by spiking a similar matrix (e.g., filtered water with added sediment and plant matter as an approximation of stormwater) with known numbers of particles of a range of sizes, morphologies, and polymers. Ideally, this particle mixture mimics what you anticipate seeing in your environmental matrix of interest. There is not currently a standard of acceptable recovery; laboratories should establish acceptable recovery limits during study design, and recoveries of spiked samples should be reported.
4.3. Reporting Results

Because there are not yet standard methods for analysis of microplastics in environmental media, reporting results in clear and consistent ways is especially important for general usability of data. Cowger et al. [109] have recently developed harmonized reporting guidelines for microplastic studies in environmental and laboratory settings, which should increase the validity, reproducibility, and comparability of microplastic studies. In addition to following those guidelines, we recommend providing particle counts rather than or in addition to total mass or volume, which is frequently used for macro (larger than 5 mm) trash reporting. Current analytical methods lend themselves to counts, and this way of reporting will be easier to link with toxicity studies, as it more easily allows calculation of concentrations of specific types of particles. Regardless of how one chooses to report data, we recommend providing sufficient information so others can convert to other commonly used units. For example, sediment microplastic concentrations have been reported as counts per dry mass, per wet mass, and per surface area, so reporting sediment water content and sampled surface area of the sediment bed is an easy way to increase data comparability.

Linking toxicity studies to real-world exposures will also require understanding environmental data; currently, monitoring studies often do not mention a lower nor an upper size limit, or only mention the targeted size class [33]. We recommend clearly reporting both sieve sizes and particle size measurements, if possible.

4.3.1. Compiling Data

The use of a data template system to report results by the analyzing laboratory is an important method of reducing various error rates and easing data compilation and analysis. Based on our experience, we recommend developing a specific electronic data deliverable template (EDD) for all studies, especially in the case of larger collaborations among institutions. This template should define the worksheets, columns and their data types, required columns, and valid values for each submission, though the specifics of such a template may differ based on project needs.

An EDD format should be developed as part of the early study design process so that required pieces of information can be identified, and expectations can be synchronized between study participants. Where possible, a limited set of vocabulary should be defined as valid for key variables. For example, it may be useful to have a color key so laboratory staff do not accidentally introduce new, overlapping color categories. For other variables, such as polymer identification, an agreed upon set of current values is a helpful starting point, but needs to be flexible to accommodate potential changes or additions. The more restrictive the EDD design, the fewer data entry, transcription, and vocabulary errors will occur, but this must be weighed against initial setup costs and ease of use/data entry.

Generally speaking, we would recommend that data be recorded on a per particle basis, with descriptive characteristics maintained separately, allowing for a variety of aggregations to be performed in the data analysis phase, as well as keeping the valid value list for each parameter more limited. For instance, a single particle should be identified as black, acrylic, and a fragment, with a width of 0.9 mm, with each piece of information recorded in separate columns, rather than as a black acrylic fragment <1 mm in a single column. For final reporting purposes as a concentration/abundance, a single aggregation can be selected.
4.3.2. Evaluation of Blanks

Laboratory and field blanks may often show significant contamination, so blank results should be reported alongside field sample results. Field blank particle counts can be compared to field sample counts in different ways; as of yet, there are no standard methods for qualifying or adjusting field results based on blank results [110].

Many researchers choose to blank-subtract, but this may not be a robust means for correcting for background levels if the procedural contamination observed in the field blanks is variable and intermittent. For this reason, we generally chose not to blank correct. Instead, we used the field and laboratory blanks to develop data qualification threshold values to provide an index of the uncertainty of the data. For this study, we used the average of the field and laboratory blanks plus two times the blank standard deviation to provide a threshold for data qualification; for values below thresholds, we provided a caveat for the results indicating the potential for significant influence from background contamination. This was a conservative approach, indicating values below these thresholds may be strongly influenced by background contamination during sample collection and analysis. This is similar to the commonly used limits of detection/quantification (LODs/LOQs) to quantify uncertainty in the study of chemical environmental contaminants, and proposed for use in microplastic research by Bråte et al. [111]. LODs give the lowest concentration where detection is possible, while LOQs are values that exhibit a greater probability of being a true quantitative value and not a random fluctuation of the blank.

4.3.3. Standardized Reporting Categories and Vocabulary

Microparticles and microplastics come in a range of shapes/morphologies. Particles are commonly classified into different shape categories, which in some cases provide insights as to the potential source of individual particles [18,112,113]. Morphology categories and definitions are not standardized, which can make comparisons between studies difficult. We used the following categories:

- **Fragment** — irregularly-shaped particle; may come from the breakdown of larger plastic debris;
- **Sphere/Pellet** — hard, rounded, or spherical particle; may come from microbeads intentionally added to consumer products or pelletized plastic preproduction material (note that in environments with more of these types of particles, these should be split into separate categories);
- **Film** — thin plane; may come from the breakdown of film-like debris, such as plastic bags and wraps;
- **Foam** — lightweight, sponge-like particle; may come from the breakdown of foam plastic debris; and
- **Fiber** — thin or fibrous particle (sometimes referred to as microfibers); may come from textiles as well as fishing gear and cigarette filters.

In some cases, several fibers cannot be disentangled, leading to an additional particle type category, **fiber bundle**. Individual fibers within a bundle may be of similar or differing chemical composition and color. This category can be grouped with fibers when relatively few fiber bundles are observed.

Previous studies have used many different shape and morphology categories, hindering comparison with the wider literature. As illustrated above, categories used for reporting should be clearly described and distinguished from one another. More categories have been suggested as a way to support source apportioning [114,115]. For example, one may choose to separate spheres and pellets, or have a separate category specifically for rubbery fragments suspected to be from tires [116]. Proposal of new categories should include an explanation for why splitting the category is important, as well as how the new category fits within older larger categories so that microparticle counts can still be compared. At a minimum, we recommend fiber counts be clearly distinguished, as many studies exclude fibers due to
their ubiquitous nature and high blank contamination rates, making total count comparisons between studies difficult.

It is critical to develop consistent vocabulary to describe observations accurately and communicate study results effectively. As discussed above, vocabulary is used inconsistently in the microplastics literature. Even the term microplastics can mean different things to different researchers. As noted previously, we propose using microparticles to describe particles less than 5 mm that are visually identified as potentially plastic, and microplastics only to describe the subset of microparticles that have been confirmed to be plastic through spectroscopy or other techniques. We prefer the operational size limit of 5 mm, as it easily distinguishes plastic particles that are regulated as trash from as yet unregulated smaller particles. Size bins are operational in nature, but should be clearly defined within each study and consistently used as much as is feasible. We recorded both operational size (sieve size) and measured size, to increase comparability and information. A consistent vocabulary of shapes/morphologies, sizes, colors, and material types should be developed and applied consistently.

The vocabulary used to describe plastic polymers and other materials identified based on spectroscopic analyses presents further challenges. At present, there is not an agreed upon vocabulary, especially for how to group plastic polymers into larger categories. We developed the categories and definitions outlined in Table 2, drawing particular guidance from previous work by Primpke et al. [107]. This is likely to be an evolving list, as plastic use in society evolves and changes. Plastic items are frequently made of a wide variety of different polymers and other materials such as metals, paper, pigments, inks, and adhesives [117], so more copolymers will probably be observed as material identification methods improve. Plastic categories may need refinement in environments in which different materials are more abundant.

Not all microparticles are microplastics, and the material of some particles cannot be definitively identified using current techniques. Unfortunately, spectroscopic identification of the chemical composition of particles is not always possible; the presence of a chemical such as a dye can mask the spectrum of the underlying material and prevent identification. In these cases, a microparticle could be identified as being of anthropogenic origin (i.e., manufactured) based on the presence of the dye, but not necessarily classified as plastic. We refer to such particles as anthropogenic unknown. In other cases, sufficient spectral information allows confirmation that the dyed particle is plastic, but cannot indicate the specific type of plastic. We refer to such particles as anthropogenic synthetic. The presence of dyes can also reduce the ability to distinguish between non-plastic materials for some particles. Particles may also have a signal for a synthetic dye and signal for a non-synthetic material such as cellulose/cotton, leading to the additional nonplastic category anthropogenic cellulosic (e.g., cotton, rayon, modal, or Lyocell). Some dyes are primarily used for cellulosic materials and can also be used to place particles in this category, and new efforts being developed to address this challenge [118]. Categories of non-plastic anthropogenic particles identified should be reported alongside definitively plastic particles to help inform source identification and management strategies, as well as potential risk assessment.

4.3.4. Comparison Between Studies

Due to the diversity of microplastics, as well as variations in study design, sampling methods, analysis methods, and reporting, comparisons between studies can be challenging. Care should be taken when comparing results from studies using different sampling and extraction techniques. Similarly, comparison among studies with and without additional polymer identification methods should be avoided due to the potential for misidentification of microplastics using solely visual techniques,
particularly for particles less than 500 μm [119]. Sieve sizes for all studies should be noted, as more microparticles will be collected when using smaller sieves.

For all studies, but especially those on organisms, extra care should be taken to minimize the introduction of additional variables, as each variable is an additional possible source of variation. The study objective will drive whether comparisons between species are valid. For example, it makes sense to compare different trophic levels or feeding habits only if the goal is to assess these variables, as species with vastly different food sources will have different probabilities of accumulating microplastics through their diet. For comparisons between populations of the same species, other differences between the populations should be minimized; for example, comparing animals of different sizes may not be valid unless an objective is to understand differences between individuals of different ages/sizes. Comparisons between studies should also only include studies in which the same tissues were collected and similar methods were used for microparticle extraction. Comparing whole fish concentrations and gut concentrations is not valid because microplastics can translocate out of the digestive tract and into other tissues [120–123]. Likewise, studies that simply rinse the lumen of the gastrointestinal tract rather than chemically extract the tissue may underestimate accumulation if particles are entrenched in the tissue.

Finally, measured microplastic abundance in biota cannot yet be linked with potential impacts. The impacts of microplastics to organisms likely depend on the microplastic size, shape, polymer type, and associated chemical mixtures, yet the vast majority of toxicological effects studies have been acute and have used high concentrations of virgin microplastic spheres of a single polymer type. In the environment, organisms experience chronic exposures to a complex mixture of weathered microplastics of multiple morphologies and chemical characteristics. Most types of organisms have not been well studied, and important questions remain even for the most well studied taxa. Simplifying all microplastics as a single contaminant makes it even more difficult to predict ecological impacts.

4.3.5. Advanced Data Analysis

Identifying the amount and types of microplastics present in environmental samples is only the first step in gaining understanding of the sources and fate of microplastics in the environment and identifying effective mitigation actions. Comparison between different types of samples and data incorporation into models is also necessary. We recommend starting with a conceptual model, which can help one develop specific hypotheses and select the right statistical analyses and modeling options. Our conceptual model of microplastics entering San Francisco Bay included construction of a conceptual model of major pathways of microplastic pollution alongside a comprehensive review of likely sources to urban stormwater runoff and treated wastewater discharges. This framework led us to perform principal component analyses (PCA) comparing the different sampled matrices to pinpoint potential sources, and drove development and application of numerical models to estimate the dispersal and fate of microparticles and microplastics in San Francisco Bay and adjacent National Marine Sanctuaries. These advanced data analyses came with their own challenges and lessons learned.

Compared with chemical contaminant data, microplastic data are tricky to work with. As with all microplastics data, how to deal with blank counts is an important consideration. There is no single “best” way to process the data at present, and appropriate processing depends on the application. For most of our data analysis, we developed thresholds for data qualification specific to each matrix and each particle morphology using standard calculations based on the average of the field and laboratory blanks and blank variability (see above). However, we decided the best practice for our transport modeling was to take a route that introduced the least amount of bias by blank subtracting, rather than the most
conservative route of reporting all data. We therefore recommend making all data available, such that the raw data (including counts from blank samples) are preserved for researchers to use based on their study goals and best judgement. In order to avoid duplicated efforts and false starts, we recommend development of a guide on how to work with environmental microplastics monitoring data, including best practices for treatment of blanks.

Our analysis of microparticles in San Francisco Bay indicates that particle material identification is one of the most important lines of evidence for identifying potential sources and assessing potential fate. While material is the most important variable to consider, other particle characteristics such as morphology and color can also help identify sources. Others have also used the properties of individual particles, including polymer type, other chemical components, color, morphology, and size to help elucidate microplastic sources [68]. However, this approach has so far provided only a limited level of source-related information, and requires more evidence of direct links between particle characteristics and sources [124].

Polymer identification can sometimes tie a particle to a specific type of plastic use and source, and it is also essential for modeling transport and fate of microparticles in the environment. Our modeling showed the fate of microparticles, in terms of retention or export from an estuarine setting like San Francisco Bay, was largely dependent on their buoyancy [51]. This dependence makes laboratory analysis, whether by density separation or by spectroscopic identification, essential in understanding the fate of microparticles entering estuarine waters.

Most previous microplastic transport modeling has focused on global-scale circulation and the transport of surface-bound, buoyant particles (e.g., [125]); however, only a fully three-dimensional analysis of transport can capture the breadth of relevant mechanisms [126]. Our model is one of the first three-dimensional, microplastic transport models to span estuarine and coastal scales. The model successfully reproduced broad spatial patterns observed in surface water and sediment samples, and demonstrated the potential for physical processes to lead to variations in particle abundance at finer scales. A particle tracking approach to the modeling allowed flexible analysis of model outputs after the fact. This avoided the need to have all field data in hand before modeling could commence, and enabled multiple analyses (e.g., with and without fibers) with a single simulation. Even though the particle tracking approach required some degree of averaging in time and space, spatial gradients were still evident in much of the model domain for both wet and dry seasons. While it remains an open challenge to predict microparticle abundance across the spatial scales and heterogeneity of San Francisco Bay, the present modeling provides a solid foundation for designing future monitoring activities, interpreting field data, and refining future mechanistic modeling efforts.

Data collection and analysis was a considerable effort, but so was bringing the data and numerical models together. We included a modeler as part of the study team from the beginning, so that many aspects of the study design were formulated with modeling in mind. For example, we would not necessarily have chosen to increase the number of samples near hydrodynamic constrictions (e.g., the Golden Gate) without knowing it would improve the modeling total flux estimates to have these data. We were also able to intentionally time surface water sampling relative to tides in order to decrease ambiguity when comparing sampling data and modeling results.

However, there were still several things we wished we had done differently when we began working with the data. First, differences in the measured size ranges for the loading data (stormwater and wastewater) versus the ambient data (surface water and sediment) made direct comparison tricky. Second, while the stormwater samples allowed us to evaluate how land-use influences loads, it is unclear
how much particle attrition or degradation occurs between urban creeks and the Bay. This would not have changed the stormwater sampling sites, but it presents another source of uncertainty when assessing model results. Third, we treated sinking particles as bed load in the model rather than attempting to incorporate particle deposition, and this may be an over-simplification. From sediment data we know that deposition occurs, but few data exist to constrain microplastic deposition and resuspension processes. Further research on deposition dynamics (i.e., does a plastic particle sinking at 1 mm/s deposit to the bed the same way as a sediment grain with the same settling velocity?) is needed. Similarly, particles at the very surface of stormwater may have been under-sampled with our methods, and as a result may not have been adequately captured in the modeling.

5. Looking Forward: Next Steps in Studying Environmental Microplastics

Microplastic pollution is a complex global issue and its management will require multi-stakeholder participation. Accurate measures of the sources, sinks, and reservoirs of microplastics in the environment are necessary to form an understanding of the magnitude of the problem, identify the highest priorities for mitigation, and evaluate the effectiveness of management strategies.

Methods for characterizing microplastic contamination are rapidly evolving and critical gaps remain. Current sample collection methods may not provide accurate characterization of environmental loads of microplastics, especially fibers, due to their ubiquitous presence as background contamination, as well as the uncertainty associated with the efficacy of sampling methods for catching microfibers [37–39]. In addition, underestimates of microplastic occurrence are likely because many methods are not able to capture smaller size classes [55]; smaller particles may be especially important for evaluation of potential toxicity impacts [9–16]. Development of reliable, standardized methods to capture fibers and smaller microplastics is necessary.

A more urgent need in the field is standard methods for QA/QC, including collecting and reporting field and laboratory blanks and accounting for evidence of background contamination in field samples. A variety of methods to acknowledge and account for background contamination are reported in the literature, and this lack of standardization inhibits accurate calculation of occurrence, as well as cross-study comparisons. Improved understanding of the sources of background contamination in the field and laboratory may inform the selection of a reporting method, and would aid in identification of additional measures to reduce background contamination.

A myriad of possible sources of microplastics to the environment have been identified. Some of these sources have been evaluated in the literature with respect to their potential to contribute to plastic pollution to marine ecosystems (e.g., [42]), but many others have not been thoroughly characterized. Improved information regarding the relative contributions of sources of microplastics is particularly useful to regional stakeholders attempting to identify effective solutions to address microplastic contamination. In particular, sources of microplastics to urban stormwater are not yet well understood. Our study indicates that urban stormwater delivers a much larger load of microplastics to San Francisco Bay than wastewater, highlighting a need for better understanding of microplastics in urban runoff. Identifying the relative contribution of secondary microplastics formed from the breakdown of larger trash will also be important for this effort. Recent studies suggest that airborne deposition may be a significant pathway of microplastic contamination in urban areas [127–130], especially for fibers, and warrants further study.

Once microplastics are released into water bodies, our modeling showed that their fate is largely determined by their rising/settling rate. A number of studies have evaluated the settling rates of virgin
plastics in the lab, and some studies have begun to look at the effect of particle weathering or biofouling on sinking rates, but to our knowledge, no one has yet measured sinking rates of recovered particles. While the extraction process often will not leave biofilms intact, even post-digestion rising/settling rates would be useful data to better inform modeling.

Finally, the likely effects of microplastics on wildlife are unknown. The majority of microplastics found in fish and bivalves in the Bay were fibers, consistent with findings from around the world. While there are studies identifying impacts of microplastic exposure to organisms, most have used only virgin plastic spheres and exposures above ecologically relevant concentrations. There is an urgent need for ecotoxicological studies that evaluate the effects of microplastics at environmentally relevant concentrations in organisms at multiple life stages [10]. However, even with more ecotoxicological data, establishing risk thresholds will be difficult, given the diversity of microplastic sizes, morphologies, and chemistries. Furthermore, initial evidence suggests that accumulation and toxicity of microplastics and other chemicals can be significantly different when organisms are exposed to mixtures rather than individual contaminants [131–137]. A robust and comprehensive evaluation of risk to aquatic wildlife may require more sophisticated approaches than currently available.

6. Conclusions

Our study of microplastics in San Francisco Bay was one of the first to conduct a comprehensive and integrated assessment using quality assurance approaches adapted from the field of trace environmental chemistry. Microplastic concentrations and characteristics throughout the Bay and surrounding ocean were assessed in five different environmental matrices (stormwater, treated wastewater effluent, surface water, sediment, and biota).

This comprehensive and pioneering assessment of microplastics in San Francisco Bay and adjacent National Marine Sanctuaries provided an ideal learning opportunity for designing and implementing microplastic research. This project was a prime example of the importance of using study questions to drive the design and selection of sampling sites, sampling and laboratory methods, and QA/QC. One of the most formidable challenges in this study was the lack of established methods for field sampling, laboratory analyses, and reporting. The lessons learned in this study provide a foundation for others to build upon as they embark on studies of this important global contaminant.

The scientific information, tools, and recommended solutions developed via recent San Francisco Bay microplastics monitoring are intended to catalyze similar efforts to understand and reduce plastic pollution around the globe. Future studies will continue to improve our understanding of microplastic pollution and track the effectiveness of management strategies. Sound science will provide a foundation for society to move forward to identify and implement successful solutions.

7. Acknowledgements

The San Francisco Bay Microplastics Project was primarily funded by the Gordon and Betty Moore Foundation, with additional financial support from the Regional Monitoring Program for Water Quality in San Francisco Bay, Patagonia, the Virginia Wellington Cabot Foundation, East Bay Municipal Utility District, the City of Palo Alto, and the California Ocean Protection Council.

8. References


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59. Magnusson, K.; Norén, F. Screening of microplastic particles in and downstream a wastewater treatment plant; IVL Swedish Environmental Research Institute, 2014.


<table>
<thead>
<tr>
<th>Study Objective</th>
<th>Scientific Needs to Adequately Address Objective</th>
</tr>
</thead>
</table>
| **Conduct a comprehensive evaluation of microplastics in Bay and adjacent National Marine Sanctuaries surface water, as well as Bay sediment and Bay fish and bivalves** | **Represent the overall condition of the Bay and sanctuaries (e.g., sufficient number of sites)**  
Represent spatial variation (e.g., mid-Bay and nearshore or “margins” sample sites); include a less urban reference site | **Represent current conditions (e.g., surface sediments instead of sediment cores)**  
Collect water samples during wet and dry seasons to evaluate seasonal influence | **Microparticle analysis must include polymer identification (for source attribution and tracking trends)**  
Use laboratory blanks and clean laboratory practices  
Demonstrate good method recoveries using spiked samples  
Minimize chemical digestion methods when possible |
| **Characterize microplastics in stormwater runoff and treated wastewater effluent discharges to the Bay to develop** | **Select higher-flow sites (e.g., larger streams or wastewater treatment facilities)**  
For stormwater, | **Focus on larger particles (i.e., not nanoparticles) that are more easily tied back to sources**  
Use standard sieve mesh sizes for comparison among | **Microparticle analysis must include polymer identification (source attribution)**  
Consider using pyrolysis GC-MS to identify** |
baseline information, assess relative loads, and identify unique sources

| Select sites with varying land-use patterns to allow extrapolation of loads using available models |
| For wastewater, select facilities with different treatment types |
| For wastewater, avoid weekend variations from weekday flows by sampling only Tuesday–Friday |

Assess uptake of microplastics into the food web and identify areas of high concern for biota

| Select spatially diverse sampling sites, including a less urban reference site |
| Select species with high site fidelity and known feeding habits and predation |
| Sample multiple matrices at the same site (sediment, water, and biota) |
| Determine appropriate backup sites should biota not be found at the original site of interest |

Develop an estuarine-marine microplastic transport model linking the transport of microplastics from the Bay out the Golden Gate to adjacent National Marine Sanctuaries

| Represent the overall condition of the Bay and Sanctuaries |
| Coordinate with model needs (e.g., locate some sample sites near model boundary conditions) |
| Sample multiple matrices at the same site |

| Consider tidal fronts and currents; water dynamics can concentrate buoyant and semibuoyant material, causing significant small-scale spatial variation, and convergence zones tend to aggregate biological nutrients and microplastics |

| Different pathways and load calculations |
| Develop storm criteria to ensure consistency and avoid sampling base flow |
| For stormwater, use depth-integrated sampling |

| Develop storm criteria to ensure consistency and avoid sampling base flow |
| For stormwater, use depth-integrated sampling |

| Polymer if monitoring urban areas that will have tire wear particles |
| Use consistent sieves across matrices to facilitate evaluation of transport |

| Microplastic analysis should include chemical identification (to inform future risk assessment) |
| Focus on the full gastrointestinal tract to assess ingestion; digest whole gut (rather than just rinse gut lumen) to capture entrenched particles |

| Assess smaller size fractions (less than 150 µm) in addition to larger standard operational size fractions; these particles have the potential to translocate out of the gut and bioaccumulate |

| Microparticle analysis must include polymer identification (to estimate buoyancy) |
| Microparticle analysis should include size measurements (to estimate buoyancy) |

Assess uptake of microplastics into the food web and identify areas of high concern for biota

| Target sufficient numbers of individuals to determine a representative level of contamination in biota |
| Collect individuals of similar size to avoid extra variables such as age |
| If compositing, collect enough individuals for replicate composites at each site |

| Sample multiple matrices at the same site (sediment, water, and biota) |
| Determine appropriate backup sites should biota not be found at the original site of interest |

| Represent the overall condition of the Bay and Sanctuaries |
| Coordinate with model needs (e.g., locate some sample sites near model boundary conditions) |
| Sample multiple matrices at the same site |
site (e.g., sediment and water)

**Table 2.** Material categories for microparticles now included in the California Environmental Data Exchange Network (CEDEN).

<table>
<thead>
<tr>
<th>Plastic polymers primarily observed in the Bay</th>
<th>Particles that may or may not be plastic, and should not be counted in conservative estimates of microplastic loads</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Anthropogenic synthetic — interpretation of Raman or FTIR spectrum indicates the material is plastic (e.g., due to the presence of a dye or additive used in plastics and not likely to be found in non-plastic materials), but does not indicate which polymer is present</td>
<td>• Anthropogenic unknown — evidence indicates the material is anthropogenic in origin, frequently due to the presence of a color (i.e., not clear or white) or the spectrum of dyes or other synthetic compounds; the underlying material may or may not be plastic</td>
</tr>
<tr>
<td>• Acrylic — a broad class including Polyacrylonitrile, Polyacrylamide, Polymethacrylate</td>
<td>• Unknown — spectroscopy is inconclusive</td>
</tr>
<tr>
<td>• Cellulose acetate</td>
<td>• Unknown potentially rubber — black, rubbery fragment with spectrum of carbon black or similar; while carbon black is used as a filler in vehicle tire rubber, this spectrum cannot be considered an exclusive marker for rubber</td>
</tr>
<tr>
<td>• Nylon (also known as polyamide)</td>
<td>Non-plastic particles detected in the Bay</td>
</tr>
<tr>
<td>• Polycarbonate</td>
<td>• Anthropogenic cellulosic — evidence indicates the material is anthropogenic in origin, frequently due to the presence of a color (i.e., not clear or white) or the spectrum of dyes or other synthetic compounds; the underlying material is cellulosic</td>
</tr>
<tr>
<td>• Polyethylene — including high density and low density polyethylenes as well as polyethylene wax; separately listed copolymers include:</td>
<td>• Anthropogenic protein – can include silk and wool</td>
</tr>
<tr>
<td>o Polyethylene co-acrylic acid</td>
<td>• Asphalt</td>
</tr>
<tr>
<td>o Ethylene/vinyl acetate copolymer</td>
<td>• Cellulosic — specific identification is not possible for these undyed particles, but they may be made of cotton, rayon, modal, or Lyocell.</td>
</tr>
<tr>
<td>o Polyethylene/polypropylene copolymer</td>
<td>• Cotton</td>
</tr>
<tr>
<td>• Polyester (fibers) and Polyethylene terephthalate (PET; non-fiber microplastics); separately listed copolymer:</td>
<td>• Glass</td>
</tr>
<tr>
<td>o Polyethylene terephthalate/polyurethane</td>
<td>• Inorganic natural material</td>
</tr>
<tr>
<td>• Polypropylene</td>
<td>• Organic natural material</td>
</tr>
<tr>
<td>• Polystyrene; separately listed copolymers include:</td>
<td>• Paint</td>
</tr>
<tr>
<td>o Polystyrene/acrylic copolymer</td>
<td>• Silicone</td>
</tr>
<tr>
<td>o Acrylonitrile butadiene styrene</td>
<td>• Stearates, lubricants, waxes</td>
</tr>
<tr>
<td>o Styrene copolymer (multiple)</td>
<td>• Wool</td>
</tr>
<tr>
<td>• Polyurethane</td>
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<tr>
<td>• Polyvinyl acetate</td>
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<tr>
<td>• Polyvinyl alcohol</td>
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<td>• Polyvinyl butyral</td>
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<tr>
<td>• Polyvinyl chloride</td>
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</tbody>
</table>
- Polyvinyl ether; separately listed copolymer:
  - Methyl vinyl ether copolymers
- Rubber — a combination of natural (isoprene) and synthetic (e.g., styrene-butadiene) polymers

Far smaller numbers of microplastics were identified as:
- Fluoroelastomer and Polytetrafluoroethylene – both fluorine-containing polymers
- Phenolic resin
- Poly(aryletherketone)
- Polyacrolein
- Polycaprolactone
- Polyether block amide
- Polyethylenimine

Figure 1. Study sites for San Francisco Bay microplastics assessment (additional surface water sites were located in the adjacent National Marine Sanctuaries and are not shown here). Trawl samples include surface water trawls performed during wet and dry seasons. Only watersheds sampled for stormwater are pictured.
Graphical abstract

Quality Assurance/Quality Control (QA/QC)

- Study Design
  - Site selection
- Collecting
  - Sampling methods
- Analyzing
  - Identity confirmation
- Reporting
  - Standard vocabulary
CRediT authorship contribution statement

CRediT (Contributor Roles Taxonomy) author statement for manuscript *Recommended Best Practices for Collecting, Analyzing, and Reporting Microplastics in Environmental Media: Lessons Learned from Comprehensive Monitoring of San Francisco Bay*

See: https://www.elsevier.com/authors/journal-authors/policies-and-ethics/credit-author-statement

Ezra Miller: Conceptualization, Investigation, Writing – Original Draft, Writing – Review and Editing, Visualization

Meg Sedlak: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – Original Draft, Writing – Review and Editing

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Christopher Holleman: Conceptualization, Methodology, Software, Formal analysis, Investigation, Writing – Review and Editing

Chelsea M. Rochman: Conceptualization, Methodology, Validation, Resources, Data Curation, Writing – Review and Editing, Supervision

Rebecca Sutton: Conceptualization, Methodology, Formal analysis, Investigation, Resources, Writing – Review and Editing, Supervision, Project administration, Funding acquisition
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐ The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:
Highlights

Highlights for manuscript *Recommended Best Practices for Collecting, Analyzing, and Reporting Microplastics in Environmental Media: Lessons Learned from Comprehensive Monitoring of San Francisco Bay*

- Lessons from a comprehensive regional study of microplastics in San Francisco Bay.
- We offer recommendations on collection, analysis, and reporting in different media.
- Study questions should drive design and site selection, methods, and QA/QC.
- Standardized QA/QC, secondary confirmation, and reporting practices are needed.