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Microparticles, Microplastics, and PAHs in Bivalves in San Francisco Bay

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Executive Summary

California mussels (*Mytilus californianus* and hybrid *Mytilus galloprovincialis* / *Mytilus trossulus*) and Asian clams (*Corbicula fluminea*) were collected at multiple sites in San Francisco Bay. Mussels from a reference area with minimal urban influence were also deployed in cages for 90 days at multiple sites within the Bay prior to collection.

Mussels from the reference time zero site, Bodega Head, had some of the lowest microparticle levels found in this study, along with resident clams from the San Joaquin and Sacramento Rivers and mussels transplanted to Pinole Point. The highest concentrations of microparticles were in mussels transplanted to Redwood Creek and Coyote Creek.

Over 98% of the particles found in bivalves were fibers. Non-fiber particles were relatively evenly split between fragments, spheres, and films. No foam particles were found. Sixteen percent of the fibers found in bivalves underwent Raman spectroscopic analysis to determine their composition. Of these, 52% were not plastic (made of natural-based material like cotton or wool; mostly of anthropogenic origin), and 24% were confirmed to be plastic, with the most common polymers being acrylic and cellulose acetate. The remaining 24% could not be identified because dyes interfered with the spectra.

Clams had lower average microparticle and microplastic concentrations than mussels, but it is unclear whether this difference reflects differences between the species' ingestion rates or differences between sampling sites (*Mytilus spp.* prefer higher salinity water than *C. fluminea*, so the two species are not found at the same sites). Similarly, microparticle abundances were significantly higher in 90-day transplant mussels compared to residents, but these sites were also different and therefore not directly comparable.

Levels of microparticles in bivalves in the Bay were comparable to those observed in bivalves worldwide, although the present study used a larger sieve size than other studies, and is therefore likely an underestimate of microparticle concentrations.

Mussel composites from six sites were also analyzed for 77 polycyclic aromatic hydrocarbon (PAH) analytes using high resolution gas chromatography / low resolution mass

spectrometry (HRGC / LRMS). The relatively small number of samples limits comparisons, but there was no clear correlation between microplastic concentrations and PAH concentrations in bivalves. It is likely that the observed PAH concentrations in bivalves reflect broader trends in PAH concentrations in the Bay rather than exposure via ingestion of PAH-sorbed microplastics.

The results of this study and current literature indicate that bivalves may not be good status and trends indicators of microplastic concentrations in the Bay unless the interest is in human health exposure via contaminated bivalve consumption.

Introduction and Objectives

High abundances of anthropogenic microparticles, including microplastics, have been found in San Francisco Bay water, sediment, and prey fish (Sutton et al., 2019, 2016), indicating a need to evaluate more broadly the accumulation of microplastics and associated chemical contaminants in the Bay food web. Bivalves are an important part of the Bay food web, linking benthic and pelagic systems (Durand, 2015). Bivalves may therefore represent a significant route for microplastics to enter the Bay food web, as they have been shown to do in other locations (Farrell and Nelson, 2013), and cause negative impacts to bivalve predators (Rochman et al., 2017). Bivalves are also frequently consumed by humans, and may represent a higher microplastics exposure risk than other seafood because they are eaten whole (Barboza et al., 2018; Smith et al., 2018). These traits suggest bivalves are a good potential candidate as a bioindicator for monitoring microplastics in the aquatic environment (Li et al., 2019; Su et al., 2018), and specifically the Bay.

Filter feeders such as bivalves are widely used for biomonitoring, as they can act as integrators of contaminants in the water column. Bivalves have been widely used in long-term monitoring programs in California such as the Regional Monitoring Program for Water Quality in San Francisco Bay (RMP), NOAA's Mussel Watch Program, and the California State Mussel Watch Program.

Measurement of contaminant concentrations in transplanted bivalves accumulated during dry season deployment is designed to provide long-term data on the bioaccumulation of chemical pollutants in biota throughout the Bay (Hardin et al., 2005). Contaminant bioaccumulation in transplanted bivalves is measured by collecting bivalves from sites that are known to have low contaminant concentrations and transplanting them to moorings located throughout the Bay. This technique has been used by the RMP for status and trends monitoring for contaminants such as polychlorinated biphenyls (PCBs) and polycyclic aromatic hydrocarbons (PAHs) since 1993.

The primary goal of this study was to characterize microplastics in transplanted and resident bivalves collected in San Francisco Bay by leveraging mussels that were being collected for PAH and nutrients monitoring. This study was designed primarily to assess whether bivalves would be a good matrix for future status and trends monitoring for microplastics.

A secondary goal was to compare microplastic and PAH concentrations to assess microplastics as a potential vector of hydrophobic organic contaminants in the Bay. In urban areas such as the Bay Area, PAHs can originate from a variety of sources, including thermal combustion processes (e.g., cooking and heating oils, coal burning), vehicular emissions (e.g., automobiles, trucks, machinery), and biomass burning (e.g., fireplaces, controlled burns, wildfires). These contaminants generally occur as complex mixtures and not as single compounds. Many PAHs have mutagenic and genotoxic potential, so it is important to whether microplastics may act as an exposure pathway of these contaminants to aquatic organisms.

Hydrophobic organic contaminants such as PCBs and PAHs readily sorb to plastic in the aquatic environment, and microplastics can therefore act as a transport medium and source of hydrophobic organic contaminants to aquatic organisms (Bergmann et al., 2015; Rochman, 2016; USEPA, 2016; Wang et al., 2018). The importance of exposure via microplastics relative to other exposure pathways depends on the species and environment, although for many species it is likely responsible for only a small fraction of total exposure (Koelmans et al., 2016). The type of plastic is also important; for example, polystyrene and polyethylene appear to more strongly sorb PAHs than polypropylene, polyvinyl chloride, or polyethylene terephthalate (Rochman et al., 2013).

Many studies in the literature identify microparticles that appear to be plastic using only visual microscopy techniques. However, not all particles that appear to be plastic are actually plastic. Microplastics are a subset of microparticles that have been definitively determined as plastic through spectroscopy or other means. In this report, we refer to particles identified visually as microparticles; particles that have been confirmed to be plastic through spectroscopy are referred to as microplastics.

This study sought to accomplish the following objectives:

1. **Quantify the abundance and characteristics of microparticles and microplastics in bivalves in San Francisco Bay.**

   Data are essential to understanding the impacts to and resilience of San Francisco Bay and its adjacent ocean areas. Monitoring trends and measuring the efficacy of management actions requires knowledge of microplastic baseline abundance, type, and

composition. Microplastics have been found in bivalves worldwide, but San Francisco Bay bivalves have not been previously assessed for microparticles or microplastics. This project aimed to establish baseline quantities and characteristics of microparticles and microplastics in bivalves to inform future monitoring efforts (i.e., to assess whether bivalves would be a good organism for future microplastic status and trends monitoring).

2. **Compare microplastic and PAH concentrations in bivalves from the same locations.**

   PAHs are a concern and target of monitoring in San Francisco Bay. This study sought to add to our understanding of the potential indirect effects of microplastics on Bay aquatic wildlife by comparing tissue concentrations of microplastics and PAHs, which plastics may help transport.

Methods

Site Selection

Sample collection for this project leveraged other bivalve monitoring efforts. Sample sites (Figure 1; Table 1) were therefore chosen to match RMP Status and Trends Monitoring sites and Nutrient Management Strategy algal toxin monitoring sites.

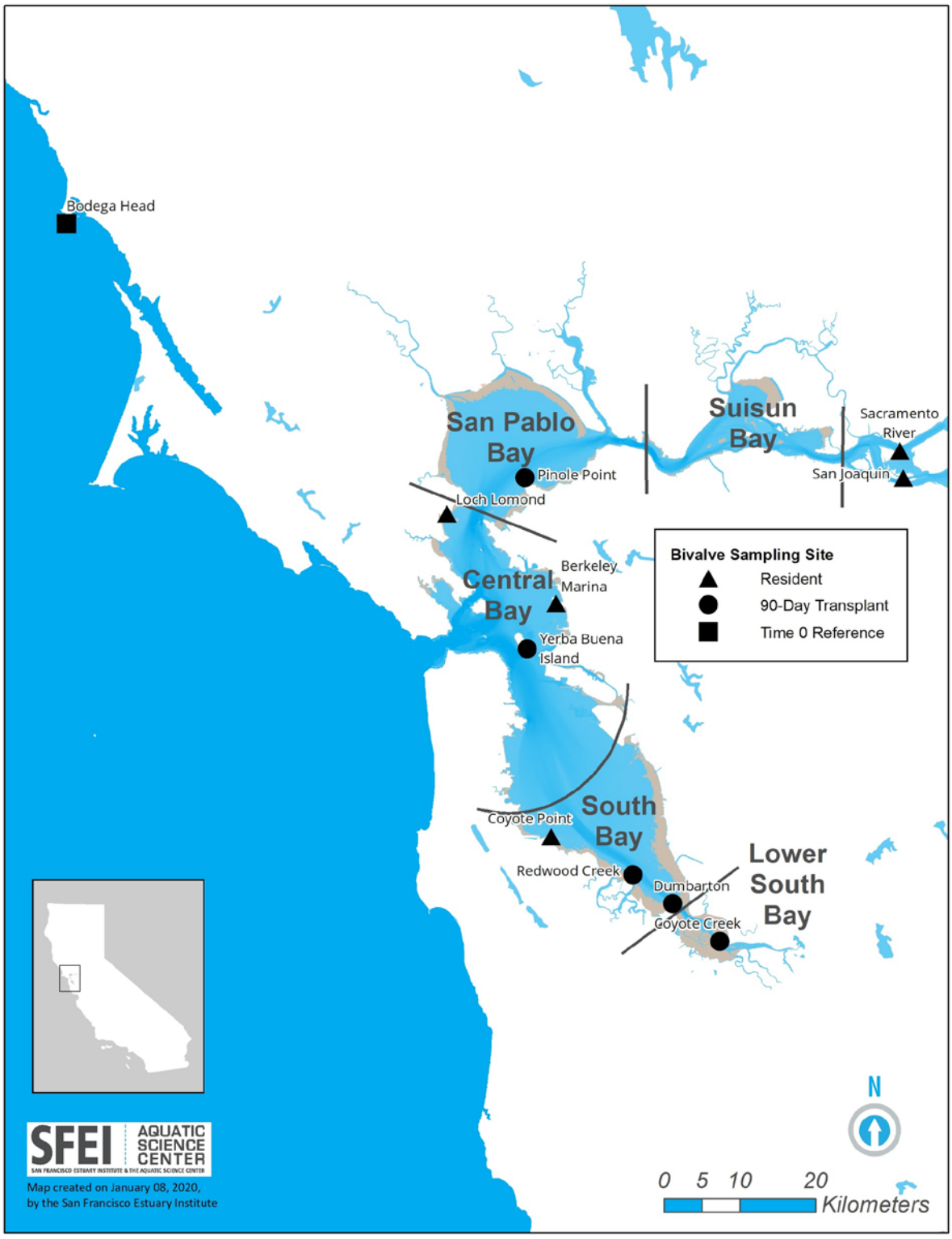

Figure 1. Bivalve sample collection sites. At the 90-Day Transplant sites (circles), mussels collected from the Bodega Head time zero (T0) reference site were deployed in cages. Bivalves collected from resident sites were *Mytilus spp.* (estuarine sites) or *Corbicula fluminea* (river sites).

Table 1. Sample collection sites.

Site Name	Site Type	Species	Latitude	Longitude	Site ID	Embayment	Co-located PAH site?
Pinole Point	90-Day Transplant	*Mytilus californianus*	38.01667	-122.3675	BD30	San Pablo	
Dumbarton	90-Day Transplant	*Mytilus californianus*	37.51377	-122.1349	BA30	South	
Yerba Buena Island	90-Day Transplant	*Mytilus californianus*	37.81392	-122.35873	BC10	Central	Y
Coyote Creek	90-Day Transplant	*Mytilus californianus*	37.46983	-122.06383	BA10	Lower South	Y
Redwood Creek	90-Day Transplant	*Mytilus californianus*	37.5470	-122.1950	BA40	South	Y
San Joaquin River	Resident	*Corbicula fluminea*	38.02362	-121.80048	BG30	Delta	Y
Berkeley Marina	Resident	*Mytilus spp.**	37.86754	-122.3176	BMM	Central	
Sacramento River	Resident	*Corbicula fluminea*	38.05570	-121.80593	BG20	Delta	Y
Coyote Point	Resident	*Mytilus spp.**	37.59083	-122.3180	CP	Central	
Loch Lomond	Resident	*Mytilus spp.**	37.97190	-122.4839	LL	San Pablo	
Bodega Head	Time 0 Reference	*Mytilus californianus*	38.30482	-123.06534	T-0 Bodega	n/a	Y

* likely a hybrid of *M. galloprovincialis* and *M. trossulus*

Sample Collection

Mytilus californianus were collected from Bodega Head on June 15, 2018, and stored in filtered seawater tanks located at the Bodega Marine Laboratory (BML) until their deployment. During this depuration period, BML Aquatic Resources Group (ARG) personnel implemented a cleaning protocol to remove fouling organisms from each mussel to minimize potential for

transfer of non-resident species from Bodega coast to San Francisco Bay. At each 90-day transplant site, 200 individuals of *M. californianus* collected from Bodega Head were deployed in two cages, each with four compartments holding 25 mussels each. Cages were hung in the water column approximately 0.3-1 m above the seafloor. At Dumbarton and Coyote Creek (sites BA10 and BA30), an additional cage containing 100 mussels was deployed to account for high mortality often exhibited at South Bay sites. These cages were attached to moorings on July 17-18, 2018. Additionally, approximately 100 mussels from Bodega Head were kept as time zero (T0) time point samples. Transplanted and resident bivalves were collected between October 22 and November 2, 2018. At the Sacramento and San Joaquin river sites (BG20 and BG30), the water was not saline enough to support *M. californianus*, so *Corbicula fluminea* were collected from the sediment bed at these sites instead. Resident *Mytilus spp.* were collected from the sides of existing structures at Berkeley Marina, Coyote Point, and Loch Lomond (sites BMM, CP, and LL) during the same timeframe.

Up to 300 bivalves were recovered/collected from each site. All bivalves were allocated for analyses in the field immediately after sample retrieval/collection. Twelve to thirty individuals from each site (depending on size) were allocated for microplastics analysis. At each site, bivalves were placed in three different foil packs (double wrapped), and then immediately frozen on dry ice and returned to temporary storage in laboratory freezers before being shipped to the University of Toronto for microparticle extraction and analysis.

Approximately 100 individuals from each PAH analysis site were allocated for chemical analysis by SGS AXYS. At each of these sites, unrinsed organisms were wrapped in two layers of aluminum foil, placed in zip-top bags, and immediately frozen on dry ice and returned to AMS for temporary storage in laboratory freezers. AMS then shipped all frozen samples to SGS AXYS for processing and chemical analysis in two shipments, November 5 and 14, 2018.

Field blanks were not collected for this study.

Microparticle Extraction and Analysis

In the microplastics laboratory, samples were thawed and rinsed with reverse osmosis (RO) water. The soft tissue was dissected out and rinsed with RO water. To digest the samples,

the soft tissue of each 4–10-bivalve composite sample was placed in a plastic cup with approximately three times the total tissue sample volume of KOH (200g/L; minimum 15 mL/container) with the lid loosely capped, and the containers were placed in the fume hood for ~10 days. After the ~10 day digestion, each sample was sieved through 125 μm and 25 μm stacked sieves, and then the >125 size fraction and the 25-125 size fraction were vacuum filtered through a 10 μm polycarbonate filter. Particles from the >125 size fraction were identified under a dissecting microscope. Particles in the smaller size fraction were not counted or characterized due to time and budget constraints.

Laboratory blanks comprised of RO water were collected for every eight samples; four laboratory blanks were analyzed. The RO water underwent the same process as a true sample. A plastic cup was filled halfway with RO water (halfway because the dissected bivalves usually filled about half the container) and then the lid was loosely capped and the container was placed in the fume hood for ~10 days (to mimic digestion time of the tissue). After the ~10 days, the blank was removed from the fume hood and the RO water was sieved and filtered using vacuum filtration, then particles from the >125 size fraction were identified under a dissecting microscope.

Approximately 18% of all identified microparticles underwent Raman spectroscopic analysis to determine the chemical composition of each particle using a reference spectra library. In an attempt to provide robust characterization by color and shape, the laboratory analyzed approximately 10% of each colour/shape category found. These particles were randomly selected after blank subtracting for the average of each morphology and color (all were lab-blank subtracted, and the transplanted samples were additionally T0-subtracted). Subsampling for spectroscopic analysis was the only instance in which blank and T0 subtraction was performed in this manner; final counts provided by the laboratory were not blank subtracted. For fibers, it was frequently difficult to discern the composition of the material due to spectral interferences from dyes. In these instances, the fibers were classified as “anthropogenic unknown base.”

Data Analysis

There is no standard method for accounting for blank contamination when reporting results in environmental assessments of microplastics. The RMP has previously chosen to report

blank results alongside field sample results. However, due to the use of composite samples in this study, reporting blank results alongside field results is not always possible. Thus, both uncorrected and blank-corrected microplastic values are reported below.

Laboratory blank results for microplastics are reported alongside field sample results. The laboratory blanks were used to develop conservative data qualification reporting thresholds for each particle shape, calculated as the average laboratory blank plus two times the standard deviation, below which results are qualified. Qualified values should be treated with caution because they may be strongly influenced by contamination from processing and analysis. The laboratory blank data as well as the threshold values are reported so individual readers can make their own assessment.

Because each sample was a composite of multiple individuals, but not all composites were the same size (composites ranged from 4 to 10 individuals), the data qualification threshold could not be converted to a per individual unit. Therefore, bivalve concentrations were corrected by the data qualification threshold when sample concentrations were converted to estimated concentrations per individual.

Comparisons were analyzed using two-sample t-tests assuming unequal variances. Statistical evaluations were considered significant at $p < 0.05$.

PAH Extraction and Analysis

PAH analysis was performed by SGS AXYS using high resolution gas chromatography / low resolution mass spectrometry (HRGC / LRMS) in accordance with SGS AXYS Method MLA-021: Analytical Method for the Determination of Polycyclic Aromatic Hydrocarbons (PAH), Alkylated Polycyclic Aromatic Hydrocarbons, and Alkanes.

For individual PAHs, the concentration at each field site was blank-subtracted if the analyte was detected in the laboratory blank (42 out of 77 analytes; Table 2). This is different from how the RMP generally deals with blank contamination; field sample results are qualified if the analyte was found in the blank, and anything less than three times the concentration found in the lab blank is deemed unreportable. All analytes were more than three times the lab blank concentration. The total PAH concentration for each site was obtained by summing the detected

concentrations of all 77 reported PAHs at each site, treating non-detects as 0. To compare with previous PAH monitoring (e.g., Oros et al., 2007), the concentrations of a subset of the 25 parent compounds were also summed (Table 2).

Table 2. PAHs measured in bivalves.

Analyte	Blank Contamination	Previous Monitoring
Acenaphthene		Y
Acenaphthenes, C1-		
Acenaphthylene		Y
Anthracene		Y
Benz(a)anthracene		Y
Benz(a)anthracenes/Chrysenes, C1-	Y	
Benz(a)anthracenes/Chrysenes, C2-		
Benz(a)anthracenes/Chrysenes, C3-		
Benz(a)anthracenes/Chrysenes, C4-		
Benzo(a)pyrene		Y
Benzo(b)fluoranthene		Y
Benzo(b/j/k)fluoranthene		Y (k only)
Benzo(e)pyrene		Y
Benzo(g,h,i)perylene		Y
Benzo(j/k)fluoranthene		Y (k only)
Benzofluoranthenes/Benzopyrenes, C1-		
Benzofluoranthenes/Benzopyrenes, C2-		
Biphenyl	Y	Y
Biphenyls, C1-	Y	
Biphenyls, C2-	Y	
Chrysene	Y	Y
Dibenz(a,h)anthracene		Y

Dibenzothiophene		Y
Dibenzothiophenes, C1-	Y	
Dibenzothiophenes, C2-	Y	
Dibenzothiophenes, C3-	Y	
Dibenzothiophenes, C4-	Y	
Dimethylchrysene, 5,9-		
Dimethyldibenzothiophene, 2,4-		
Dimethylfluorene, 1,7-		
Dimethylnaphthalene, 1,2-		
Dimethylnaphthalene, 2,6-	Y	Y
Dimethylphenanthrene, 1,7-		
Dimethylphenanthrene, 1,8-		
Dimethylphenanthrene, 2,6-	Y	
Dimethylphenanthrene, 3,6-		
Fluoranthene	Y	Y
Fluoranthene/Pyrenes, C1-	Y	
Fluoranthenes/Pyrenes, C2-	Y	
Fluoranthenes/Pyrenes, C3-		
Fluoranthenes/Pyrenes, C4-		
Fluorene	Y	Y
Fluorenes, C1-	Y	
Fluorenes, C2-	Y	
Fluorenes, C3-	Y	
Indeno(1,2,3-c,d)pyrene		Y
Methylanthracene, 2-		
Methylbenzo(a)pyrene, 7-		
Methylchrysene, 1-		
Methylchrysene, 5/6-		

Methyldibenzothiophenes, 2/3-	Y	
Methylfluoranthene, 3-/Benzo(a)fluorene	Y	
Methylfluorene, 2-	Y	
Methylnaphthalene, 1-	Y	Y
Methylnaphthalene, 2-	Y	Y
Methylphenanthrene, 1-	Y	Y
Methylphenanthrene, 2-	Y	
Methylphenanthrene, 3-	Y	
Methylphenanthrene, 9/4-	Y	
Naphthalene	Y	Y
Naphthalenes, C1-	Y	
Naphthalenes, C2-	Y	
Naphthalenes, C3-	Y	
Naphthalenes, C4-	Y	
Perylene	Y	Y
Phenanthrene	Y	Y
Phenanthrene/Anthracene, C1-	Y	
Phenanthrene/Anthracene, C2-	Y	
Phenanthrene/Anthracene, C3-	Y	
Phenanthrene/Anthracene, C4-	Y	
Pyrene	Y	Y
Retene	Y	
Tetramethylnaphthalene, 1,4,6,7-		
Trimethylnaphthalene, 2,3,5-	Y	Y
Trimethylnaphthalene, 2,3,6-	Y	
Trimethylphenanthrene, 1,2,6-		

Results

Quality Assurance: Laboratory Blanks

Four laboratory blanks were analyzed, with microparticle counts of 11, 13, 14, and 18, respectively. All particles in the blanks were fibers; no non-fiber particles were found in the blanks.

The laboratory blanks indicated that sample contamination can occur during processing and analysis. Because there are no standard protocols for using blank data, they were treated in the same manner as previous RMP microplastic data. Values were thus qualified when they were below a conservative data qualification threshold of the average laboratory blank plus two times the standard deviation, or 20 fibers/sample. The conservative reporting threshold applies only to fibers, as the blanks did not contain any other morphologies of microparticles. Qualified values should be treated with caution because they may be strongly influenced by contamination from processing and analysis.

Bivalve fiber concentrations were corrected by the data qualification threshold when sample concentrations were converted to concentrations per individual. We chose to correct for the threshold in this case because each sample was a composite of multiple individuals, but not all composites were the same size. This meant that the data qualification threshold could not be converted to a per individual unit to be displayed alongside the field results.

Particle Occurrence and Morphology

Of the 31 composite tissue samples analyzed, five had fiber counts below the threshold for data qualification of 20 fibers/sample, indicating that these samples may not be different from laboratory contamination. These samples included one each from Bodega Head, the Sacramento and San Joaquin Rivers, Berkeley Marina, and Pinole Point. No sampling site had multiple samples below the threshold. However, two of these sites, Pinole Point and Sacramento River, each had one sample lost in transport/processing so there were only two samples analyzed from these sites.

Of the particles found in bivalves in this study, 98% (1201 out of 1220) were fibers. Non-fiber particles (19 out of 1220) were split between fragments (10), spheres (3), and films (6). No foam particles were found in bivalve tissue. Sites in South and Lower South Bay had the highest number of non-fiber particles, but overall levels of non-fiber particles were extremely low throughout the Bay.

Bivalves from the reference T0 site, Bodega Head, had some of the lowest microparticle levels, along with resident bivalves from the San Joaquin and Sacramento Rivers and bivalves transplanted to Pinole Point (Figures 2 and 3). Transplanted bivalves had slightly higher average concentrations than residents ($p = 0.035$), but overall, resident and transplant sites had similar particle concentration distributions (Figures 2 and 3).

Relative percent differences (RPDs) between replicate samples ranged from 0 to 90%, with an average RPD across all sampling sites of 38%.

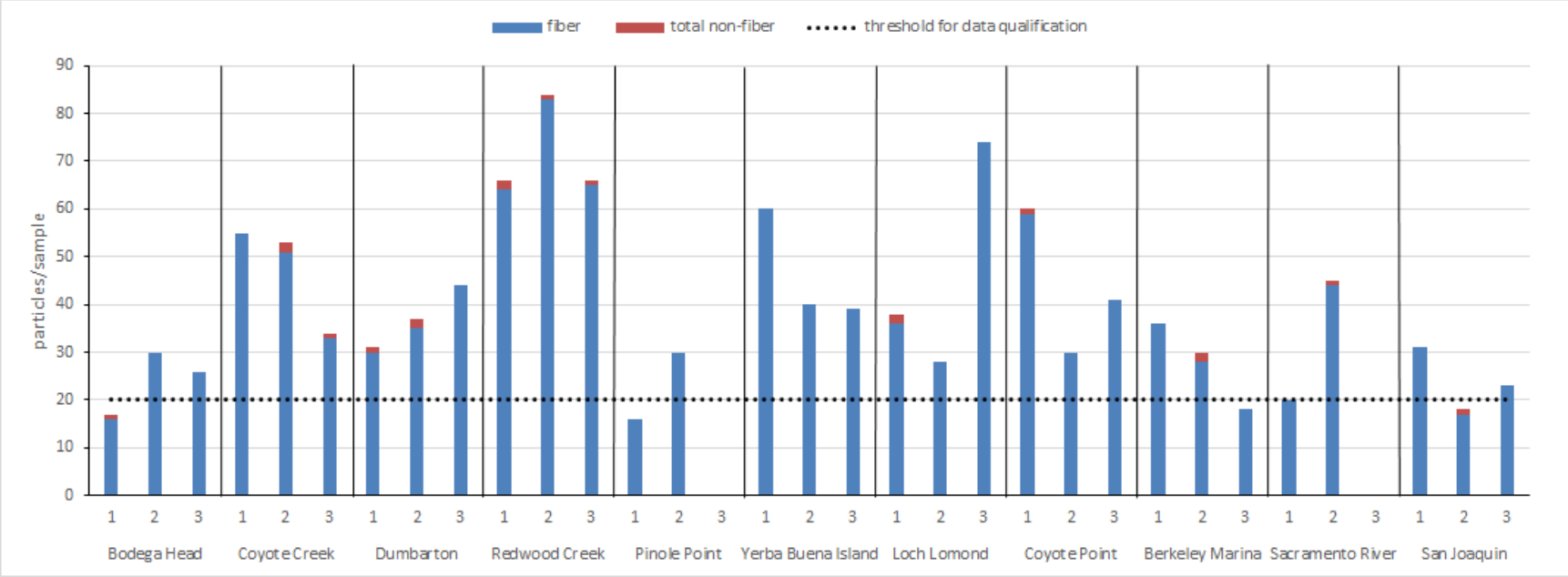

Figure 2. Microparticle abundances in bivalve tissue compared with laboratory blank contamination. Composite samples included four to ten individual bivalves. Numbers on the x-axis indicate replicates. Non-fibers include fragments, spheres, and films.

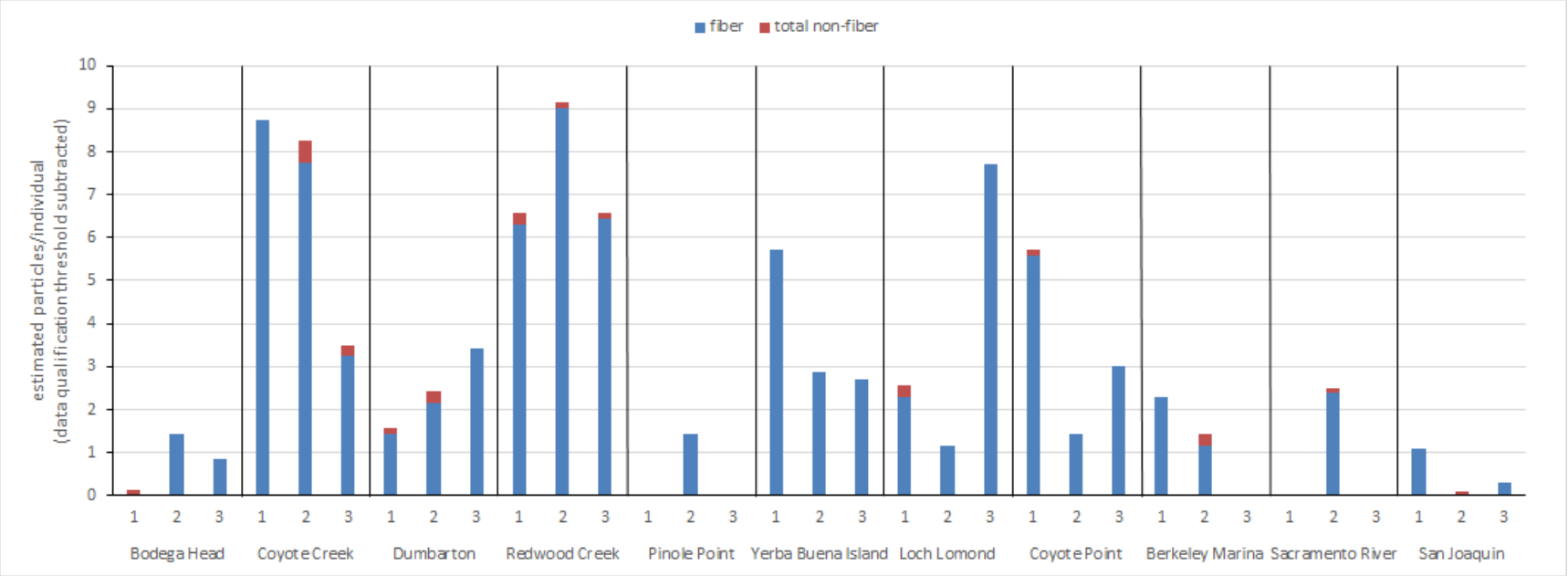

Figure 3. Estimated microparticle abundances per individual bivalve. Fiber concentrations shown have been corrected by the threshold for fiber data qualification calculated from the laboratory blanks (20 fibers/sample). Non-fibers include fragments, spheres, and films. Numbers on the x-axis indicate replicates.

Particle Composition

Out of the total 56 fibers found in blanks, 21 (38%) were analyzed by Raman spectroscopy to determine their composition (Figure 4). Of these, 10% (2) were identified as plastic, 62% (13) as natural or cellulosic material, and 24% (5) as anthropogenic unknown (i.e., dyed material that may or may not be plastic). The two fibers in the blanks that were confirmed to be plastic were black polyester and clear anthropogenic synthetic (spectroscopy identified ditridecyl adipate, a plasticizer, but not the base material). Of the natural or cellulosic fibers, the majority (10 out of 13) were identified as anthropogenic due to the presence of a dye. Most of the fibers in laboratory blanks were blue or black in color (52% and 9%, respectively), and strong Raman signals from the dyes in these fibers resulted in many of these fibers being identified as anthropogenic unknown. One microfiber could not be identified (i.e., could not be matched to any spectra in the library).

Sixteen percent of the fibers found in bivalves (196 of 1201) underwent Raman spectroscopic analysis. Of these, 52% (110) were not plastic, although the majority were of anthropogenic origin (dyed), and 24% were confirmed to be plastic, with the most common polymers being acrylic, polyester, and cellulose acetate. Twenty four percent (50) could not be identified.

Of the non-fiber particles found in bivalves, 79% (15 of 19) were analyzed using Raman spectroscopy. Of these, one was inorganic natural material (not plastic), seven could not be identified, and seven were confirmed to be plastic, with the most common polymers being polyethylene, polypropylene, and polystyrene

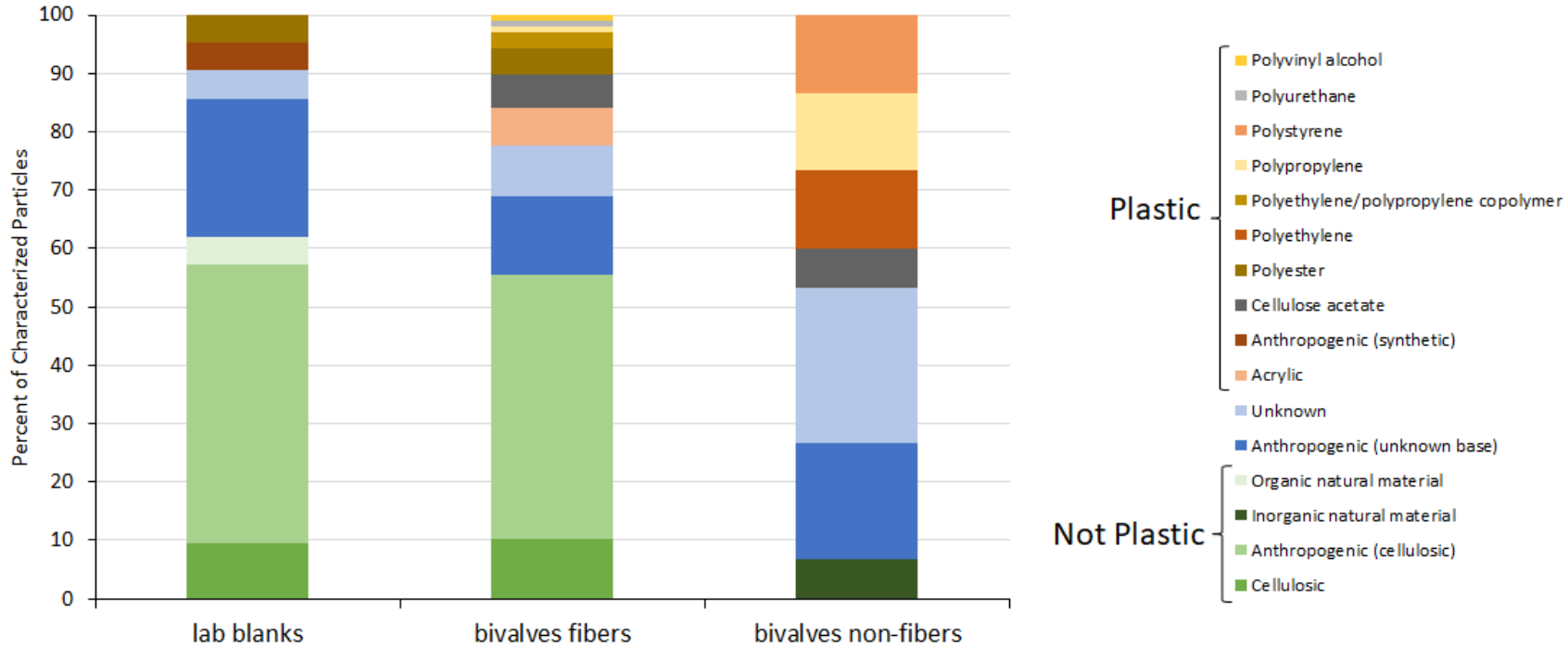

Figure 4. Raman spectroscopic identification of particles. Of the 1276 particles found in this study, 232 were characterized using Raman (38% of particles from blanks; 16% of fibers and 79% of non-fibers in field samples). Non-plastics are shown in shades of green, unknown materials in shades of blue, and confirmed plastic in shades of orange, yellow, and brown. Non-fibers include fragments, spheres, and films (no foams were found).

Discussion

Regional and Species Differences

Corbicula fluminea had lower average microparticle and microplastic concentrations than *Mytilus spp.* ($p = 0.025$ for composites and $p = 7.07\times10^{-5}$ for estimated individual concentrations). However, since the different species were not collected at the same sites or in the same parts of the Bay (*Mytilus spp.* prefer higher salinity water than *C. fluminea*), it is unclear whether this difference reflects differences between the species' ingestion and/or excretion rates or differences in microparticle abundance at the different sampling sites. *M. californianus*, *M. galloprovincialis*, and *M. trossulus* do not have major differences in feeding behavior, but were located in the water column, whereas *C. fluminea* were collected from the bed. Samples from previous work did not include both areas so we do not have an estimate of ambient concentrations.

Although previous work showed high concentrations of microparticles and microplastics in Lower South Bay sediments compared to other embayments (Sutton et al., 2019), bivalves from Lower South Bay (i.e., Coyote Creek) did not have the highest microparticle abundances. It is likely, however, that filter-feeding bivalves more closely reflect concentrations of microplastics in the water column than in the sediment. Surface water samples from Lower South Bay did not have higher microparticle abundances than other embayments, but microparticles were not assessed throughout the water column (Sutton et al., 2019).

Microparticle abundances were significantly higher in 90-day transplant bivalves compared to residents ($p = 0.035$). However, this result is difficult to interpret, as the sites where transplanted and resident samples were collected are different and therefore not directly comparable. To understand whether transplanted bivalves accumulate microparticles at a different rate than residents, transplanted individuals would need to be deployed at sites where residents can also be collected. A comparison of pre- and post- depuration concentrations of microparticles in T0 organisms would also be useful.

Assuming transplanted bivalves are comparable to residents, the higher average concentration at transplant sites may indicate higher concentrations or longer residence times of microparticles in the water column at these locations. Redwood Creek (BA40) and Coyote Creek (BA10) had the highest concentrations, suggesting microparticles may be more abundant at these sites. Redwood Creek is a priority PCB monitoring area for the RMP, which is known to be highly contaminated with organic pollutants, and may also have higher microparticle loads. However, Coyote Creek is not.

Microplastic Concentrations in Bivalves

Because only a subset of particles were spectroscopically analyzed and not all particles that underwent Raman spectroscopy were definitively identified, we estimated upper and lower bounds of the average numbers of microplastics in bivalves from Bodega Head, San Francisco Bay, and the Delta Rivers (Table 3).

The laboratory blanks did not have non-fiber microparticles, which indicated the non-fiber microparticle counts were less influenced by background contamination than the fiber counts. We therefore estimated fibers and non-fiber microplastics separately, and estimated the portion of each that were microplastics based on the results of the spectroscopic analysis.

We estimated an upper bound for the average number of microfibers per bivalve by blank-correcting microfiber counts by the average of the laboratory blanks (14 fibers/sample), and a lower bound by subtracting the conservative threshold for data qualification (20 fibers/sample). Because no non-fiber particles were found in blanks, we assumed the average observed counts were not inflated by contamination.

For the lower bound of plastic fibers, we used the percentage of fibers confirmed to be plastic. For the upper bound, we also assumed 60% of the anthropogenic unknown fibers were plastic, based on the industry estimate that approximately 60% of textiles today are made from nylon and polyester (Almroth et al., 2018).

San Francisco Bay microplastic fiber estimates are much higher than Bodega Head and Delta Rivers microplastic fiber estimates, which are roughly comparable. The non-fiber

microplastic estimates are much lower than the fiber estimates, and similar between the three locations.

There are many possible reasons why fibers dominate microparticles found in Bay bivalves. Fibers were the most common morphology found in Bay water, sediment, and fish (Sutton et al., 2019). Mussels also reject a significantly lower proportion of fibers compared to spheres (Ward et al., 2019), so they may be more likely to accumulate fibers compared to other morphologies. Additionally, modeling of microparticle transport in the Bay based on particle density indicated that many non-fiber particles are likely to sink (Sutton et al., 2019), and therefore bivalves filtering the water column are less likely to be exposed to these morphologies for a significant amount of time before they sink to the sediment. In contrast, fibers are less likely to sink, and therefore bivalves filtering the water column may be exposed to fibers for longer, increasing the likelihood of ingestion.

Table 3. Estimated microplastic concentrations per individual bivalve. Non-fibers includes fragments, spheres, and foams.

Location	**Species**	**Fibers**	**Non-Fibers**
		(estimated microplastics/individual)	
Bodega Head	*M. californianus*	0.18–0.30	0.01–0.02
San Francisco Bay	*Mytilus spp.*	0.87–1.4	0.03–0.04
Rivers	*C. fluminea*	0.20–0.32	0.01–0.02

Microparticle Levels Compared to other Studies

Numerous studies have quantified the presence of microplastics in bivalves (Catarino et al., 2018, 2017; Cho et al., 2019; Digka et al., 2018; Li et al., 2018, 2016, 2015; Mathalon and Hill, 2014; Naji et al., 2018; Phuong et al., 2018; Qu et al., 2018; Rochman et al., 2015; S.A., 2019; Santana et al., 2016; Schessl et al., 2019; Su et al., 2018; Van Cauwenberghe et al., 2015; Van Cauwenberghe and Janssen, 2014; Zhao et al., 2018). However, comparison between studies is complicated by differences in methods, reporting, and species. There are currently no

standardized methods for analyzing microplastics in biota, meaning each study is unique in sample pre-treatment methods (e.g., storage and handling, dissection, digestion), microparticle size ranges analyzed, identification methods (e.g., visual, spectroscopy), and blank collection and interpretation. Even without these differences, differences in species and location among studies may also affect microplastic counts, as each species may have a different ability to excrete particles, and sampling season and location may also play a role in microplastic accumulation.

We compared microparticles in Bay bivalves with other studies with these methodological and species differences in mind. Many studies report microparticles per mass tissue rather than per individual; however, we were unable to compare concentrations per mass tissue because the Bay samples were not massed prior to extraction. Table 4 lists only studies that reported concentrations per individual bivalve. We compared only microparticle concentrations, and not microplastic concentration estimates, due to the range of differences in methods for plastic identification, subsampling, and reporting.

Almost all other studies used a smaller sieve size than our study, meaning Bay results represent a more conservative estimate of particle abundance compared to those made for other regions that include smaller particle size ranges. However, even though we would expect lower apparent abundances in studies like this one using larger sieve sizes, levels observed in SF Bay were comparable to those observed in most other locations. This indicates that bivalve microparticle concentrations in the Bay may be higher than in other regions. This result is consistent with the relatively high concentrations previously observed in Bay sediment and surface water (Sutton et al., 2019). However, due to differences in sampling locations between studies and the highly heterogeneous nature of microplastic concentrations in Bay water, comparisons between bivalve and environmental concentrations at specific sites are not possible.

Bivalves had a higher proportion of fibers than Bay surface water or sediment. Higher proportions of fibers compared to surface water and sediment were also observed in Bay prey fish (Sutton et al., 2019). These higher proportions of fibers in biota may be due to preferential feeding on fibers or enhanced rejection of other particle morphologies.

Table 4. Summary of microparticle counts in this study and in comparable studies around the world. Fiber counts reported for the present study have been corrected by the threshold for data qualification of 20 fibers/composite sample.

Reference	Location	Species	Common Name	Digestion/ Density Separation	Filter Pore Size (µm)	Tissue Concentration (#/individual)	Conc. Range	Dominant Morphology	ID Method	Dominant Polymer
This study	San Francisco Bay	*Mytilus spp.*	California mussel	KOH	125	3.8 ± 2.8	0 – 9.1	fibers (98%)	Raman	acrylic, polyester, cellulose acetate
		Corbicula fluminea	Asian clam			0.78 ± 1.1	0 – 2.5			
	Tomales Bay	*Mytilus californianus*	California mussel			0.76 ± 0.72	0 – 1.4			
Cho et al. 2019	South Korea (market-bought)	*Crassostrea gigas*	Pacific oyster	KOH/lithium meta-tungstate	20	0.77 ± 0.74	0 – 2.6	fragments	µFTIR	polyester
		Mytilus edulis	blue mussel			0.68 ± 0.64	0 – 2.4	fragments		polyester
		Tapes philippinarum	Manila clam			1.15 ± 0.74	0 – 2.8	fragments		polyester
		Patinopecten yessoensis	scallop			1.21 ± 0.71	0 – 2.8	fragments		polyester
Mathalon & Hill 2014	Halifax Harbor, Nova Scotia	*Mytilus edulis*	blue mussel	H2O2/NaCl	0.8	106 – 126		only assessed fibers	visual only	n/a
	west coast of Newfoundland and Labrador (aquaculture)					178				
Rochman et al. 2015	California (market-bought)	*Crassostrea gigas*	Pacific oyster	KOH	n/a	0.6 (± 0.9)	0 – 2	fibers	visual only	n/a
Phuong et al. 2017	French Atlantic coast	*Crassostrea gigas*	Pacific oyster	KOH/KI	12	0.61 ± 0.56		fragments	µFTIR	polyethylene, polypropylene
		Mytilus edulis	blue mussel			2.1 ± 1.7		fragments		
Su et al. 2018	Middle-Lower Yangtze River Basin, China	*Corbicula fluminea*	Asian clam	H2O2	20		0.4 – 5.0	fibers (60-100%)	µFTIR	polyester, polypropylene, polyethylene

Reference	Location	Species	Common Name	Digestion/ Density Separation	Filter Pore Size (µm)	Tissue Concentration (#/individual)	Conc. Range	Dominant Morphology	ID Method	Dominant Polymer
Zhao et al. 2018	Avery Point, CT	*Mytilus edulis*	blue mussel	H2O2/NaI	0.8	0.4 ± 0.6	0 – 2	fragments (48%), fibers (52%)	Raman/FTIR	polypropylene, polyester, cellulose acetate
Catarino et al. 2018	Forth River, Edinburgh, UK	*Mytilus edulis*	blue mussel	enzyme	0.8	10.4 ± 3.42 0.9 ± 0.99 1.3 ± 2.38		fibers, particles, films	FTIR	polyester, poly(ether-urethane)
Li et al. 2016	coast of China	*Mytilus edulis*	blue mussel	H2O2/NaCl	5	4.6 3.3	1.5 – 7.6	fibers, fragments	µFTIR	cellophane, polyethylene terephtalate, polyester
Li et al. 2018	coast of UK	*Mytilus edulis*	blue mussel	H2O2/NaCl	5		1.1 – 6.4	fibers, fragments	µFTIR	polyester, polypropylene, polyethylene
Lusher et al. 2017	Norwegian coast	*Mytilus spp.*	blue mussel	KOH	2.7	1.84	0 – 14.67	fibers (85%), fragments (11%)	µFTIR	cellulose-based polymers
Qu et al. 2018	coast of China	*Mytilus edulis* *Perna viridis*	blue mussel Asian green mussel	H2O2/NaCl	5		0.77 – 8.22	fibers (>80%), fragments	ATR microscopy	polyester
Davidson & Dudas 2016	Baynes Sound, British Columbia (aquaculture)	*Venerupis philippinarum*	Manila clam	HNO3	1.2	11.3 ± 6.6		fibers (90%)	visual only	n/a
Digka et al. 2018	Northern Ionian Sea (Mediterranean Sea)	*Mytilus galloprovincialis*	Mediterranean mussel	H2O2	1.2	0.9 ± 0.2	0 – 2	fragments	FTIR	polyethylene

Comparison with PAH Concentrations

PAH concentrations in bivalves were measured at six of the microplastics sites (Figure 5). Microplastic abundances did not correlate with the total PAH concentration (R^2 = 0.491; Figure 6) or with the sum of the subset of previously monitored PAHs (R^2 = 0.528).

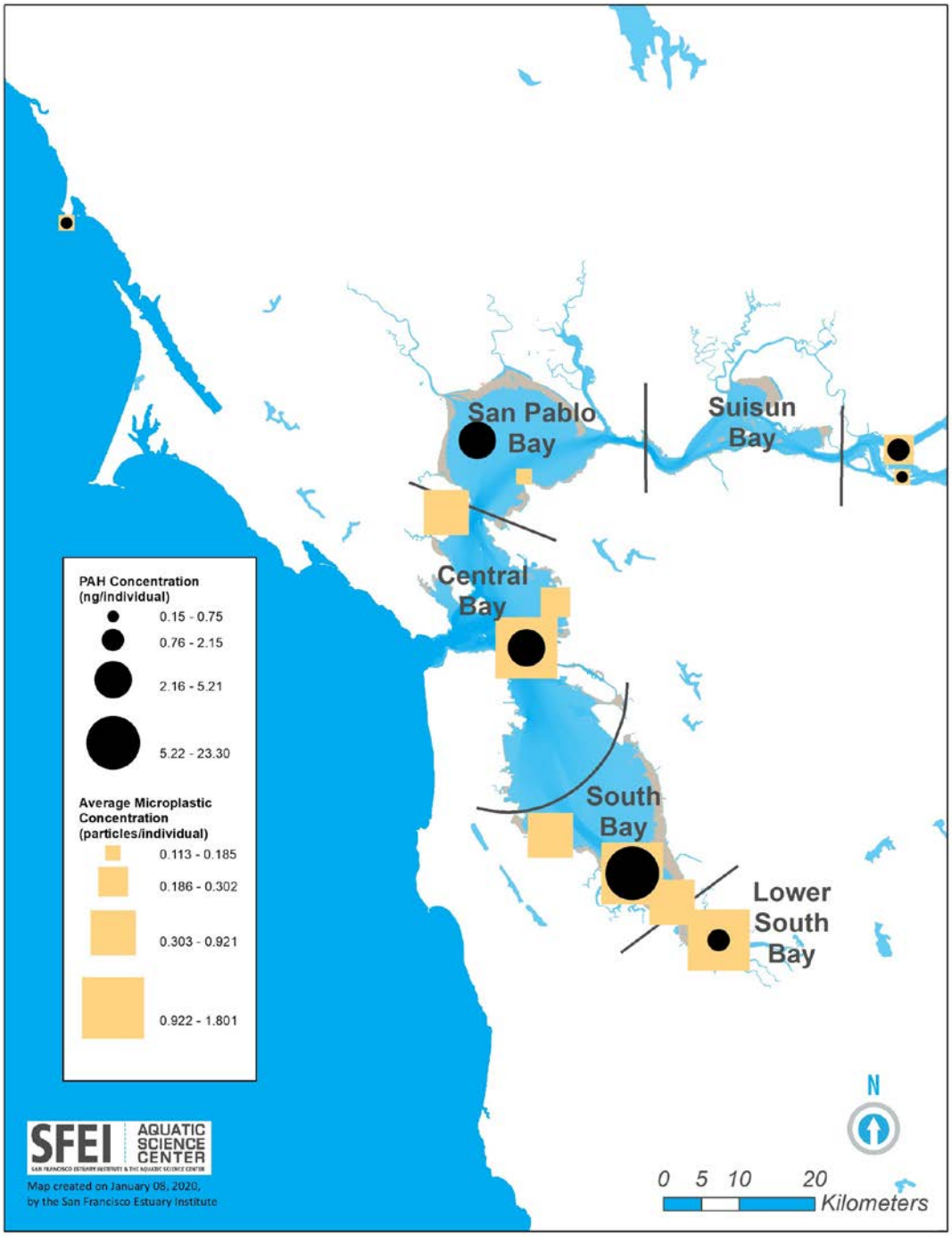

Figure 5. Total polycyclic aromatic hydrocarbon (PAH) concentrations (ng/individual) in *M. californianus* and microplastic concentrations (particles/individual) in *M. californianus* and *C. fluminea*. Total PAH concentration was calculated as the sum of 77 individual analytes (Table 2). For each individual PAH, concentrations were blank-subtracted if the analyte was detected in the blank (42 out of 77 analytes). Microplastic concentrations were estimated based on Raman spectroscopic analysis of a subset of particles and assuming 60% of anthropogenic unknown fibers are plastic, based on the industry estimate that approximately 60% of textiles today are made from nylon and polyester.

Although sources of microplastics and PAHs differ, plastic efficiently absorbs hydrophobic organic chemicals such as PAHs, meaning microplastics can sometimes act as a vector and source of hydrophobic organic contaminants to marine organisms. We therefore hypothesized that we would observe a correlation between microplastic abundances and total PAH concentrations.

While the relatively small number of samples limits our ability to compare microplastic and PAH concentrations, there was no clear correlation (Figure 6). Some sites with fewer microplastics per individual bivalve also had lower concentrations of total PAHs (e.g., Bodega Head, San Joaquin, and Sacramento River). Redwood Creek, the site with the highest PAH concentrations, also had high microplastic loads. However, Coyote Creek, had a low PAH concentration compared to the microplastic concentration found in the bivalves. This is not surprising, as Coyote Creek is currently 303(d) listed for trash impairments, and high microplastic levels are likely linked with high macroplastic trash levels. Bivalve PAH tissue concentrations were consistent with previous years' bivalve monitoring data and 2018 sediment PAH concentrations (unpublished).

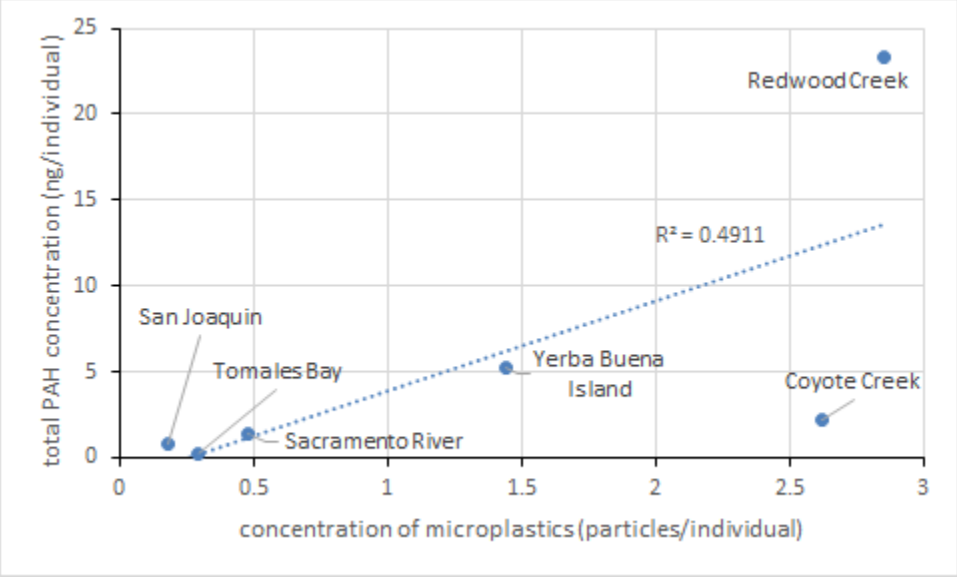

Figure 6. Total PAH concentrations in *M. californianus* compared to upper bound microplastic concentrations in *M. californianus*. Only sites where both PAH and microplastics were measured are shown.

It is likely that the observed PAH concentrations reflect broader trends in PAH concentrations in the Bay rather than exposure via microplastics, as microplastic ingestion is not

expected to be the only source of PAHs to bivalves. This result is consistent with recent modeling efforts that have shown the flux of hydrophobic organic contaminants bioaccumulated from natural prey overwhelms the flux from ingested microplastic for most habitats (Beckingham and Ghosh, 2017; Mohamed Nor and Koelmans, 2019), which implies that microplastic ingestion is not likely to significantly increase bivalve exposure to PAHs.

Use of Bivalves for Microplastic Monitoring

Bivalves have been proposed as a suitable bioindicator for microplastic pollution because of their wide distribution, important ecological niche, susceptibility to microplastic uptake, and close connection with marine predators and human health (Li et al., 2019; Su et al., 2018). Field investigations have demonstrated microplastic uptake by bivalves all over the world, with temporal and spatial variability linked to human activity (Table 4), although many of these studies are not comparable due to the lack of standardized methods and reporting.

A bioindicator species for microplastics should ingest, without bias, the majority of plastic particles to which it is exposed. Bivalves may therefore not be good microplastics bioindicators because they feed selectively and do not simply ingest all particles they capture on their gills (Ward and Shumway, 2004). Microplastic size and shape have been shown to affect the rejection, ingestion, and egestion of plastic particles by oysters and mussels (Ward et al., 2019). In particular, oysters and mussels are more likely to reject plastic spheres than plastic fibers. They are also more likely to reject larger particles.

Similarly, the ratios of microplastic morphologies found in San Francisco Bay bivalves in this study does not match the ratios of morphologies found in Bay sediment or surface water, which could indicate selective uptake of fibers or selective excretion of non-fiber morphologies. The majority of other studies also reported detecting mostly fibers in bivalve tissues (Table 4). Since bivalves evolved to reject particles naturally low in nutritional value, it is likely that they more easily reject similarly shaped microplastics such as spheres/pellets, fragments, and foams, whereas fibers may be more likely to be caught in their mucus and ingested.

Conclusions

Bivalves are an important link between benthic and pelagic systems in the Bay food web, and may be an important route of microplastic exposure to higher trophic level organisms, including humans. This study sought to quantify the abundance and characterize the type and chemical composition of microparticles in bivalves in the Bay and compare the results with studies from other regions, and with PAH contamination in co-located bivalves to determine whether microplastics appear to have a role in PAH exposure.

While all bivalves from the Bay contained microparticles, five sites each had one composite sample replicate with concentrations below the conservative data quality threshold of 20 microfibers/sample, below which laboratory contamination may be a significant component of detected microparticles. Resident *Corbicula fluminea* from the San Joaquin and Sacramento Rivers and *M. californianus* transplanted to Pinole Point, as well as the reference T0 site, Bodega Head, had some of the lowest microparticle levels found in this study. The highest concentrations of microparticles were found in *M. californianus* transplanted to Redwood Creek and Coyote Creek.

Of the particles found in bivalves, 98% were fibers. Sixteen percent of the fibers found in bivalves underwent Raman spectroscopic analysis to determine their composition. Of these, 52% were not plastic, with the majority being of anthropogenic origin, and 24% were confirmed to be plastic, with the most common polymers being acrylic, polyester, and cellulose acetate. The remaining 24% could not be identified because dyes interfered with the spectra.

We estimated concentration ranges of microplastics in bivalves using the spectroscopic results. *M. californianus* from the less urban reference site, Bodega Head, had an estimated average of 0.18–0.29 plastic fibers/individual, whereas *Mytilus spp.* in San Francisco Bay had an estimated average of 0.87–1.38 plastic fibers/individual. This result was expected, as Tomales Bay near Bodega Head also had lower levels of microplastics in sediment and prey fish.

C. fluminea from the San Joaquin and Sacramento Rivers had an estimated 0.20–0.32 plastic fibers/individual. The difference between this result and the San Francisco Bay *Mytilus*

spp. estimate of 0.87–1.38 plastic fibers/individual may reflect differences between the species' ingestion rates or differences between the areas sampled.

There was no clear correlation between microplastic concentrations and PAH concentrations in bivalves, suggesting that microplastic ingestion is not likely a major vector for bivalve exposure to PAHs in San Francisco Bay.

Although methodological differences made comparisons between studies difficult, average levels of microparticles in bivalves in San Francisco Bay were comparable to those observed in bivalves worldwide, although the levels in San Francisco Bay are likely higher than other areas, as the methods used in this study yield a more conservative estimate of microparticle concentrations. Bivalves may not be a good bioindicator for monitoring microplastics in the environment due to their selective particle ingestion.

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