Marine Pollution Bulletin 196 (2023) 115609

Contents lists available at ScienceDirect

Marine Pollution Bulletin

journal homepage: www.elsevier.com/locate/marpolbul

Microparticles in marine mussels at regional and localized scales across the Salish Sea, Washington

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ARTICLE INFO

Keywords:
Marine debris
Pollution
Anthropogenic
Shellfish
Bioindicator
Microplastic

ABSTRACT

Microparticles (MP; particles <5 mm) are ubiquitous in marine environments. Understanding MP concentrations at different spatial scales in the Salish Sea, Washington, USA, can provide insight into how ecologically and economically important species may be affected. We collected mussels across the Salish Sea at regional and localized scales, chemically processed tissue to assess MP contamination, and used visual and chemical analyses for particle identification. Throughout the Salish Sea, mussel MP concentrations averaged 0.75 ± 0.09 MP g^{-1} wet tissue. At a regional scale, we identified slight differences in concentrations and morphotypes of MP while at a localized scale these metrics were not significant and did not differ from controls. In a subset of particles, 20 % were identified as synthetic materials, which include polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), and nylon. Differences in MP sources, heterogeneous transport of MP, and distinct shellfish feeding mechanisms may contribute to plastic contamination patterns in the Salish Sea.

1. Introduction

Global plastic production and pollution have grown substantially over the last 70 years, with 460 million tonnes produced annually in 2019 (OECD, 2022). With the rise of plastic production comes the projected exponential rise of microparticle (MP) pollution (Cózar et al., 2014; Jambeck et al., 2015). Microparticles and microplastics are a prominent type of marine pollution and are ubiquitous across the globe (Thompson et al., 2004; Law and Thompson, 2014; van Sebille et al., 2015). The presence of MP in the oceans is a relatively new observation and our knowledge of potential biological implications is limited to recent studies (2004–present; Avio et al., 2017, Harris et al., 2021c). Here, we define microparticles (MPs) as a single umbrella term to encompass all small suspected anthropogenic debris (including plastic, synthetic, semi-synthetic, natural, dyed, etc.) particles between 1 μm – 5 mm in size (Arthur et al., 2009; Hartmann et al., 2019).

Due to their small size and ubiquity, MP are ingested by organisms from multiple functional groups including suspension feeders, planktivores, detritivores, and carnivores (Thompson et al., 2004; Wright et al., 2013; Frias et al., 2014; Van Cauwenberghe and Janssen, 2014; Avio et al., 2015; Palmer and Herat, 2021). Of these, suspension feeding bivalves are documented to ingest the highest quantity of MP (Setälä et al., 2016). Microparticles can negatively impact physiology (e.g. immune response, reproduction, feeding rate) in marine organisms and can subsequently have ecosystem-level impacts (Sussarellu et al., 2016; Harris and Carrington, 2019; Franzellitti et al., 2019; Pedersen et al., 2020; Harris et al., 2021a; Mendrik et al., 2021).

In coastal environments, suspension-feeding bivalves, such as mussels, have historically served as bioindicators to detect pollution (Mussel Watch, Widdows et al., 1997, De Witte et al., 2014, Lanksbury et al. 2014) and can be used as a biological metric of stressful environmental conditions (Carrington et al., 2015). Mussels are known to sequester pollution and toxics from surrounding water, making it relatively easy to estimate water contamination levels through mussel tissue analyses (Lanksbury et al. 2014). Further, mussels act as benthic-pelagic couplers, concentrating planktonic particles (including MP) into biodeposits

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https://doi.org/10.1016/j.marpolbul.2023.115609
Received 3 July 2023; Received in revised form 26 September 2023; Accepted 28 September 2023

that are dense and nutrient-rich, linking the bottom substrate (benthic) to the water column (pelagic; Harris et al., 2021a).

High concentrations of MP are likely released to the waterways in densely populated urban centers compared to less populated regions (likely due to industrial centers and subsequent transport from stormwater, wastewater, roads, etc.; Luo et al., 2019). However, it is not only population density that contributes to high MP concentrations. Microparticles may accumulate where currents deposit sediment or in basins with long residence times (where water stagnates) and disperse by rivers, currents, and fast-moving water (Wessel et al., 2016). Further, marine environments are dynamic and fluctuate spatially and temporally, as waters are continually changing with the tides, currents, and fluvial inputs (Uncles et al., 2000), so both large regional as well as small localized differences in MP concentrations may exist. Understanding biologically available MP concentrations at different spatial scales (regional and localized) may provide insight into how ecologically and economically important species living in the same region may be affected in the future.

Previous research indicates that MP concentrations vary broadly in the Salish Sea (located between Washington State, USA and British Columbia, Canada) ranging from 0.26 to 9200 particles m^{-3} (Davis III and Murphy, 2015; Desforges et al., 2015; Harris et al., 2021b). The Salish Sea is a large estuary, receiving freshwater inputs from large rivers (e.g. Duwamish and Fraser Rivers), seawater from the Pacific Ocean, and includes multiple water basins with varying water residence times. Generally, the Salish Sea has a cumulative water residence time of 327 days (MacCready et al., 2021), where basins closer to the Pacific Ocean (Juan de Fuca Strait and Whidbey Basin) have shorter residency times (1–2 months) while basins further from the Pacific Ocean (Hood Canal and South Puget Sound) have longer water residence times (1–4 months; Supplemental Table 1; Sutherland et al., 2011, Martinelli et al., 2020, MacCready et al., 2021). The Salish Sea is also home to one of the largest container terminals on the West Coast of the USA, the Port of Seattle (Port of Seattle, 2023), and is adjacent to several large cities including Tacoma and Seattle in Washington State, USA and Victoria and Vancouver in British Columbia, Canada, serving as both an ecologically and economically important region.

Many studies aiming to understand MP concentrations in marine environments focus on water and sediment, which are thought to reflect transient (in the habitat for a short time) and terminal (in the habitat for a long time) concentrations, respectively. Accurate water MP contamination assessment requires sampling large volumes, which can be resource prohibitive (e.g. access to boats, large volume seawater pump, funding, etc.), adding a level of inaccessibility for many researchers. Conversely, sediment is thought to contain relatively high MP concentrations as the eventual resting place for the majority of MP due to vertical transport and biofouling (Woodall et al., 2014; Zhang, 2017; Choy et al., 2019). Small volumes of sediment can be analyzed to accurately measure MP concentration; however, many benthic habitats are difficult to sample and are inaccessible to researchers. Overall, water and sediment sampling present two viable approaches to measuring MP concentrations across transient and terminal time; however, these sampling strategies can be costly and do not address medium-term, or biologically available, concentrations.

Suspension feeding bivalves present a way to measure biologically available MP in the water column and are reflective of medium-term concentrations. Specifically, mussels' particle processing time (filtration and ingestion) is 18–84 h on average (Kinjo et al., 2019) and they are capable of filtering approximately 24 l of water per day (Harris and Carrington, 2019), providing a cost-effective and accessible MP sampling method. Additional life history traits such as the ability to sequester particles and toxics relative to their environmental contamination, role as bioindicator, and cosmopolitan distribution suggest mussels are an advantageous method to measure MP concentration at regional and local scales.

Larger regional and smaller localized spatial MP contamination patterns may inform management plans, aquaculture, and remediation efforts in an economically and ecologically important region such as the Salish Sea. As of 2023, there is a limited number of published studies examining spatial MP concentrations of water (3), sediment (1), and organisms (5) in the region (Avery-Gomm et al., 2012, 2013; Desforges et al., 2014, 2015; Davis III and Murphy, 2015; Covernton et al., 2019; Martinelli et al., 2020; Harris et al., 2021b). All of the aforementioned studies examine extensive regional MP contamination or a time series at one location, ignoring possible small-scale localized MP contamination patterns. Tatoosh Island, an ecological research station located on Makah Land, is situated at the meeting place of the Salish Sea and Pacific Ocean and presents a unique location to study localized MP concentrations where sites are less than one kilometer apart around the shoreline circumference. The physical attributes found in the Salish Sea and Tatoosh Island, paired with mussel abundance, can provide insight into biologically available MP concentrations across regional and localized scales within the same geographical body of water.

In this study, we compare mussel MP concentrations at regional and localized scales. Regional assessment includes 10 sites in the Salish Sea, ranging from highly urbanized areas (Tacoma and Seattle) to rural areas (Neah Bay) while localized assessment includes 5 sites on Tatoosh Island, a remote island at the edge of the Pacific Ocean. Mussels, an abundant suspension feeding species, are hypothesized to ingest higher quantities of MP than other types of organisms (e.g. deposit feeders, omnivores, and herbivores; Setälä et al., 2016) and are thus expected to contain MP if present in the water column across regional, localized, urban, and rural areas. Our research aimed to assess differences and similarities in mussel MP concentrations and composition across regional and localized scales.

2. Methods

2.1. Field collection

Pacific blue mussels, *Mytilus trossulus* (shell length of 45 ± 5 mm), were collected from 11 locations with 15 total sites across the Salish Sea, Washington State, USA (Fig. 1; Supplemental Table 1). Mussels included in regional analyses were collected July – September 2018 and were collected from marinas when possible, from either the public boat launch or the furthest pier from shore (depending on availability). Marinas ranged in size and quantity of people and boat traffic (possible vectors for anthropogenic debris); therefore, the number of boat slips was considered in analyses. Moving from the south through the Salish Sea and out to the Pacific Ocean, regional sites were: Tacoma, Seattle, Kingston, Mukilteo, Coupeville, Anacortes, Friday Harbor, Port Townsend, Port Angeles, and Neah Bay (Fig. 1; Supplemental Table 1). Mussels included in local analyses were collected in May 2019 from Tatoosh Island from the intertidal during low tide (approximately +1 m above MLLW; there is no marina or boat launch on the island). Localized sites were: Main Beach, Strawberry Draw, Simon's Landing, Glacier, and North Island (Fig. 1; Supplemental Table 1). Mussels from regional and localized sites were transported in acid-washed glass jars to a laboratory at the University of Washington, Seattle campus for processing.

2.2. Tissue digestion

Ten mussels from each site were chemically digested and analyzed for microparticle (MP) contamination. Mussel shell lengths were measured, byssal threads removed, body tissue extracted from the shell and placed in aluminum weigh boats, and tissue was immediately covered with aluminum foil to reduce contamination. Mussel tissues were weighed for wet weight (g) and subsequently transferred to 1 l glass flasks for chemical digestion.

A standardized wet oxidation extraction protocol (Li et al., 2015) was used to digest mussel tissue. Each flask contained one mussel and 100 ml 30 % H_2O_2 and was covered with aluminum foil to prevent

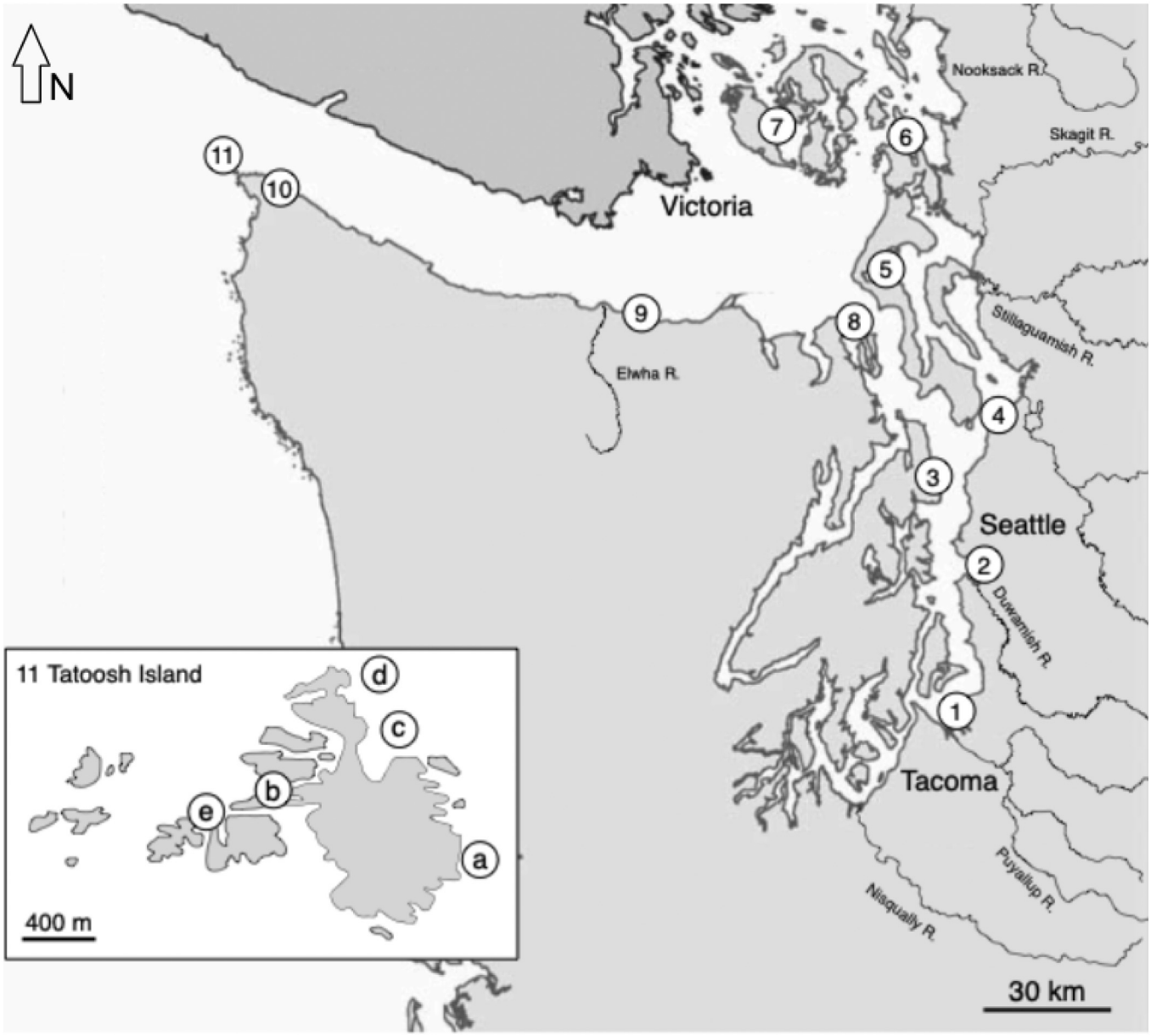

Fig. 1. Map of the Salish Sea, Washington, showing sampling sites (1−11). The site names from the South towards the Salish Sea are as follows: (1) Browns Point - Tacoma, (2) Shilshole - Seattle, (3) Kingston, (4) Mukilteo, (5) Coupeville, (6) Anacortes, (7) Friday Harbor, (8) Port Townsend, (9) Port Angeles, (10) Neah Bay, and (11) Tatoosh Island containing five sites: (a) Simon's Landing, (b) Strawberry Draw, (c) Main Beach, (d) North Island, and (e) Glacier.

contamination. Flasks were placed in an oscillating incubator at 65 °C for 24 h. Once mussel tissue was observed as dissolved (100 % of samples after 24 h), 400 ml of hyper-saturated salt solution (1.2 g cm^{-3}; lab-grade NaCl) was added and flasks stood at ambient room temperature for an additional 24 h. After samples settled, the top 200 ml of solution was vacuum filtered over a 5 μm cellulose nitrate membrane filter inside a fume hood. Each filter was subsequently rinsed with 50 ml DI water to clean the funnel and wash away the remaining H_2O_2. Filters were placed in sterile petri dishes and sealed with Parafilm. Filters were left to dry for at least seven days before visual quantification.

2.3. Contamination prevention and control

To reduce MP contamination, all equipment underwent extensive cleaning prior to sampling and processing. All glassware was soaked in an acid bath (1 M HCl), triple DI water rinsed before use, and covered with aluminum foil at all times. The hyper-saturated salt solution was filtered (0.45 μm cellulose nitrate membrane) before use. Researchers wore white 100 % cotton lab coats in the lab at all times when processing samples. A paired process control with no mussel accompanied each day of sample digestions. A clean filter (in an open petri dish) was placed on the lab counter while processing samples as an ambient control.

2.4. MP visual quantification

Dry filters were visually inspected under a compound microscope for MP. All MP were photographed using a Nikon Eclipse Ni camera and the 4× or 10× objective. During visual inspection, it is nearly impossible to determine polymer composition; therefore, all particles that appeared of anthropogenic origin were counted, categorized by morphotype and color (Fig. 2), photographed, and measured for length and width using ImageJ. Morphotypes included fibers, foils, spheres, and fragments. Microparticle color was recorded; if particles had multiple colors, only the dominant color was recorded for the purpose of our analyses. Rare

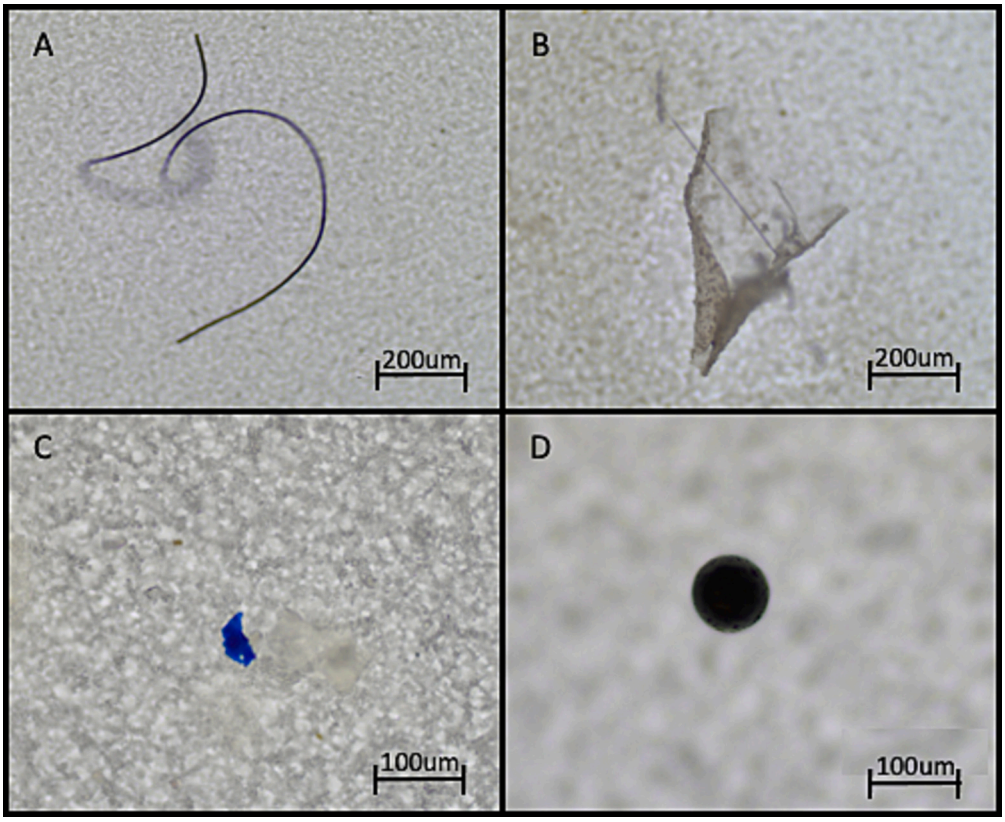

Fig. 2. Representative samples of different microparticle morphologies and colors: A) black fiber, B) clear film, C) blue fragment, D) black sphere. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

colors (<1 % of total observations) were categorized as "other."

2.5. Polymer identification

A subset of MP were picked from samples for chemical analysis with a Renishaw inVia Raman microspectrometer equipped with a 785 nm laser. Chosen MP were representative of the quantities found across regional and localized sites (grouped together), sample types (mussel and control), morphologies, and colors of particles observed. Two objectives, 10× and 50×, were used to optimize the analytical laser focus for analysis, and the laser power and acquisition times were adjusted to each particle's sensitivity to thermal damage. Following imaging, each MP's Raman spectrum was individually and manually matched to a known Raman spectrum in the Renishaw Raman Database of Polymers library, containing 267 polymer identifications. Each spectrum was also subjected to data processing depending on the signal-to-noise ratio and fluorescent interference (which produced a curved, sloped baseline). Baseline correction allows for the removal of a distorted spectrum before comparing the sample spectrum to a known reference spectrum. A match was determined when all the characteristic Raman peaks, corresponding to the main active functional groups in the polymer appeared in the sample spectrum.

2.6. Data analysis

All data analyses and graphs were made with computing software R for Mac OS X (R Core Team, 2019); version 3.6.2, Dark and Stormy Night). Level of significance was set at $\alpha < 0.05$. Regional and localized data as well as associated controls were analyzed separately because they were collected and processed in different years. Both regional and localized mussel concentrations (MP g^{-1} wet tissue) were normally distributed and homogeneity of variance was not confirmed, as determined by Bartlett and Shapiro-Wilks tests, respectively. The effect of site on the proportion of contaminated mussels (containing ≥1 MP) was evaluated with a generalized linear model (GLM) with a binomial distribution. The effect of site, basin, and fiber length on mussel MP concentration were evaluated with the Kruskal-Wallis rank sums test, a non-parametric test. Subsequent differences in mussel MP concentration between sites and between basins were evaluated by pairwise post-hoc tests (Mann-Whitney test). The effect of marine size (# of boat slips) on regional MP concentration was evaluated with Kendall-Theil nonparametric regression trend. Microparticle morphotype and color compositions were assessed with multivariate analyses of variance (MANOVA) across sites.

3. Results

3.1. Regional

Microparticles (MPs) were found in 63 % of mussels sampled in the Salish Sea, 75 % of process control filters, and 47 % of ambient control filters. A greater percentage of mussels collected from Browns Point - Tacoma and Anacortes contained MP than any other site and both types of controls (90 % for both; $p = 0.04$ for both; GLM; Fig. 3A). There were no significant differences in contamination (containing ≥1 MP) between the remaining regional sites and the control filters ($p > 0.06$; GLM).

Microparticle concentrations averaged 1.92 ± 0.19 MP per mussel (mean ± standard error; range: 0–10) and 0.75 ± 0.09 MP g^{-1} wet tissue (range: 0–3.7) across regional sites in the Salish Sea (Figs. 3B; 4). Mussel concentrations (MP $mussel^{-1}$) differed from controls ($p = 0.008$; Kruskal-Wallis; Table 1); however, only mussels collected from Coupeville had higher total MP concentrations than controls ($p < 0.05$; Mann-Whitney). When normalizing for weight (and thus not including controls) there was a difference in sites ($p = 0.005$; Kruskal-Wallis; Table 1;

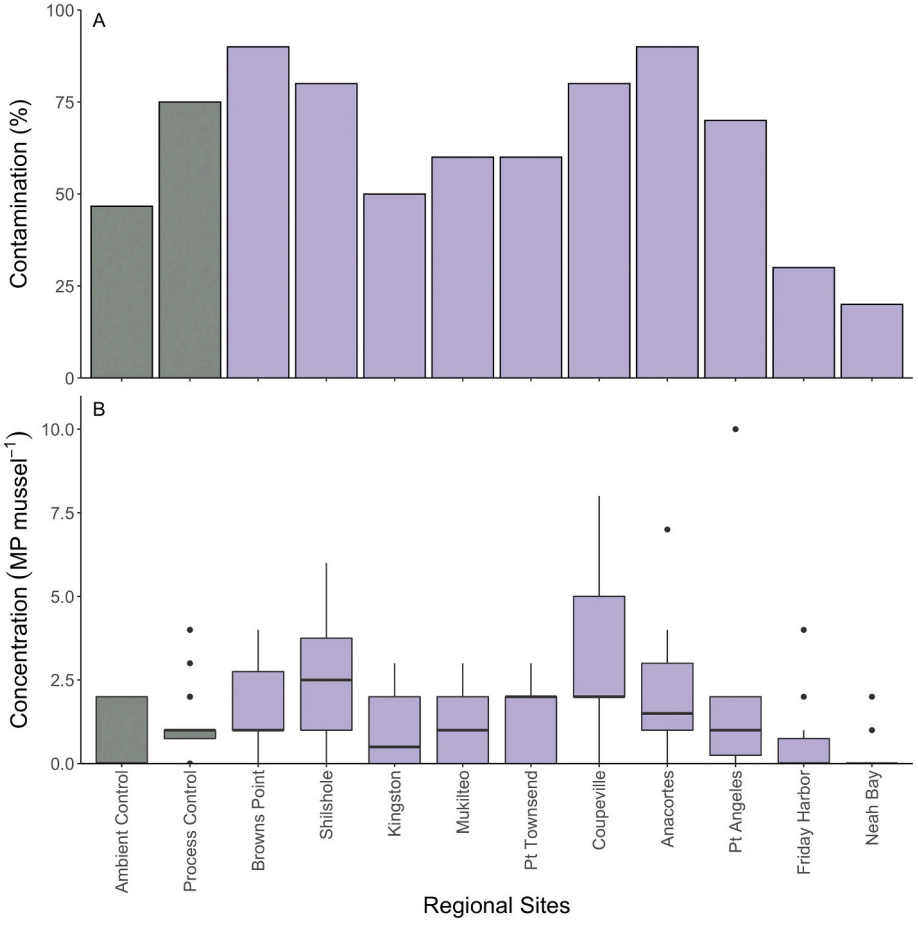

Fig. 3. Regional mussel microparticle A) contamination (containing ≥1 MP) percentage and B) concentration (MP mussel^{-1}). Ambient and process controls are on the left in grey, and sites are ordered from the South Salish Sea on the left, moving towards the Pacific Ocean on the right in purple. Boxes represent upper and lower quartiles, dots represent outliers, and solid lines within boxes represent median values. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

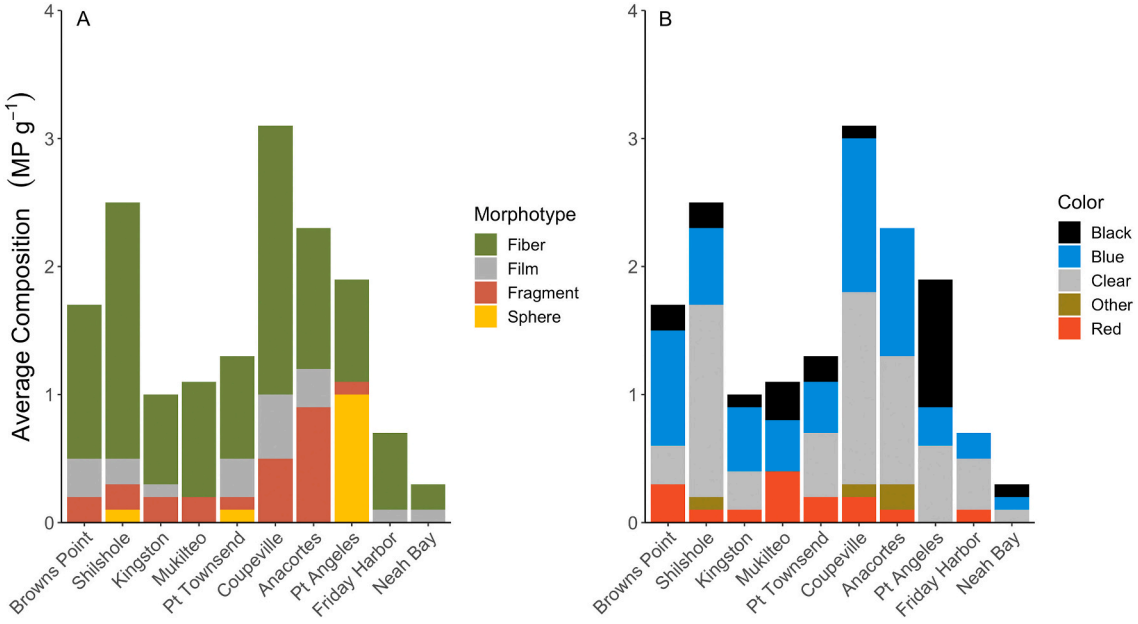

Fig. 4. Composition of average microparticle A) morphotype and B) color composition per gram of wet mussel tissue (MP g^{-1} wet tissue) across regional sites. Sites are ordered from the South Salish Sea on the left, moving towards the Pacific Ocean on the right.

Table 1
Kruskal-Wallis rank sum tests on mussel MP concentration (MP mussel^{-1}) and mussel MP concentration normalized for weight (MP g^{-1} wet tissue, not including controls) across sites. Asterisk (*) and bold indicates statistical significance ($p < 0.05$).

Metric	X^2	df	*p*-value
Regional			
MP mussel^{-1}	25.429	11	**0.008***
MP g^{-1} wet tissue	23.752	9	**0.005***
Localized			
MP mussel^{-1}	5.176	6	0.522
MP g^{-1} wet tissue	4.668	4	0.323

Fig. 4); however, only Coupeville and Neah Bay differed in MP contamination, with 1.47 ± 0.44 MP g^{-1} and 0.13 ± 0.1 MP g^{-1} wet tissue, respectively ($p = 0.02$; Mann-Whitney). Mussel MP concentration was not dependent on basin ($p = 0.08$; Kruskal-Wallis; Supplemental Fig. 1A) or the number of boat slips in the marina from which the mussels were collected ($p = 0.85$; Kendall-Theil; Supplemental Fig. 1B).

Microparticle morphologies (fiber, foil, sphere, or fragment) found in mussels (normalized by weight) differed by site (p = 0.04; MANOVA; Fig. 4A; Table 2) where fibers significantly differed across a regional scale ($p = 0.03$; MANOVA). Morphotype did not differ between control types ($p = 0.35$; MANOVA; Fig. 5A). Fibers were the dominant morphotype in all samples, accounting for 42–85 % of total MP morphologies found in mussels (Fig. 4A) and 81–91 % of total MP morphologies in control filters (Fig. 5A). Fiber lengths averaged 580 ± 43 μm (mean ± standard error) and did not differ at a regional scale or with controls ($p = 0.36$; Kruskal-Wallis; Table 3; Supplemental Fig. 2). Microparticle colors found in mussels (normalized by weight) differed by site ($p = 0.01$; MANOVA; Fig. 4B; Table 2) where clear colored MP proportions significantly differed across regional sites ($p < 0.001$; MANOVA; Fig. 4B). Color did not differ between control types ($p = 0.25$; MANOVA; Fig. 5B). The majority of MPs were either blue or clear-colored, in both mussels and control filters (74 % and 70 %, respectively).

3.2. Local

Microparticles were found in 42 % of mussels sampled on Tatoosh Island, 43 % of process control filters, and 25 % of ambient control filters. There were no differences in the percentage of mussels containing MP across sites or controls ($p > 0.15$; GLM; Fig. 6A). Microparticle concentrations averaged 0.70 ± 0.15 MP per mussel (range: 0–4) and 0.28 ± 0.06 MP g^{-1} wet tissue (range: 0–1.6) across localized sites on Tatoosh Island (Figs. 6B; 7). There was no difference in total MP concentration between mussels and control filters ($p = 0.58$; Kruskal-Wallis; Table 1) nor was there a difference when normalizing for weight (and thus not including controls; $p = 0.32$; Kruskal-Wallis; Table 1).

Microparticle morphologies found in localized Tatoosh Island mussels (normalized by weight) did not differ by site (p = 0.25; MANOVA; Fig. 7A; Table 2) nor did they differ between control types (p = 0.25; MANOVA; Fig. 5C). Fibers were the dominant morphotype in most samples, accounting for 42–100 % of MP morphologies found in mussels and 100 % of MP morphologies in the control filters. Fiber lengths averaged 611 ± 106 μm (mean ± standard error) and did not differ at a local scale or with controls ($p = 0.19$; Kruskal-Wallis; Table 3; Supplemental Fig. 2). Microparticle colors found in localized Tatoosh Island mussels (normalized by weight) did not differ by site ($p = 0.47$; MANOVA; Fig. 7B; Table 2) nor did they differ between control types (p = 0.25; MANOVA; Fig. 5B). The majority of MPs were either blue or clear-colored, in both mussels and control filters (63 % and 71 %, respectively).

Table 2
Multivariate analysis of variance (MANOVA) on microparticle morphology and color composition across sites normalized for mussel weight, therefore not including controls. Asterisk (*) and bold indicates statistical significance (p < 0.05).

	df	Num df	Den df	p-value
Regional				
Morphology	9	36	360	**0.04***
Color	9	45	450	**0.01***
Localized				
Morphology	4	8	90	0.25
Color	4	20	176	0.47

3.3. Particle identification

Approximately 14 % (35 of 245 total particles) of suspected anthropogenic MP were successfully identified by chemical analyses and spectral matching (regional and localized sites were grouped together). There was a significant amount of fluorescence interference, possibly from a layer of biofilm, that made the polymer identification process challenging. As a result, some of the initially selected MPs could not be identified through spectral matching. Seven, or 20 %, of the identified MPs were synthetic materials, which include polyethylene terephthalate (PET; $n = 2$), polypropylene (PP; $n = 1$), polystyrene (PS; n = 2), nylon (n = 1), and ink (n = 1). The MP identified as ink (blue fragment) matched a mixture of ink and plastic, but the identification of plastic could not be separated from the collected spectrum. Examples of the identified microplastics and corresponding Raman spectra are shown in Fig. 8. Approximately 48 % of visually identified MP were not anthropogenic, but instead more natural materials: 31 % of the particles were identified as minerals such as orthoclase ($n = 3$) or quartz ($n = 8$) and 17 % of the particles were graphitic materials (carbon fiber; $n = 6$). Fig. 9 illustrates the categorization of abundance, size, and identification of particles found inside mussels.

4. Discussion

Throughout the Salish Sea, mussel microparticle (MP) concentrations averaged 0.75 ± 0.09 MP g^{-1} wet tissue where fibers were the most common morphotype. At a regional scale, we identified slight differences in concentrations and morphotypes while at a localized scale these two metrics were not significant and did not differ from baseline controls. No gradient from the Salish Sea in Tacoma to the Pacific Ocean at Neah Bay was detected.

Fibers were consistently the prominent morphotype in mussels, consistent with previous studies on sea surface tows, water samples across depths, and oysters in the Salish Sea (Desforges et al., 2014; Davis III and Murphy, 2015; Martinelli et al., 2020; Harris et al., 2021b). Additionally, the average fiber length observed here, 580 ± 43 μm, was consistent with previously observed fiber lengths in the region, 606–657 μm (Desforges et al., 2014; Harris et al., 2021b).

The percentage of contaminated mussels (containing ≥1 MP) differed between two regional sampling locations and the control filters while MP concentration only differed at one regional sampling location and control filters. Localized Tatoosh sites were not different from one another or the controls. A slight, if any, difference between mussel contamination and control filters signifies that mussels generally do not contain many MP or that we had contamination in our samples during processing. The authors recommend additional contamination prevention measures in future studies, including quicker processing time (less elapsed time between mussel collection, dissection, and digestion), a clean room dedicated to MP processing (rather than shared lab space), and using a laminar flow hood (rather than fume hood).

Taken as a whole, the percentage of contaminated mussels and MP characteristics (concentration, morphotype, color, and chemical identification) were relatively consistent across the Salish Sea, similar to

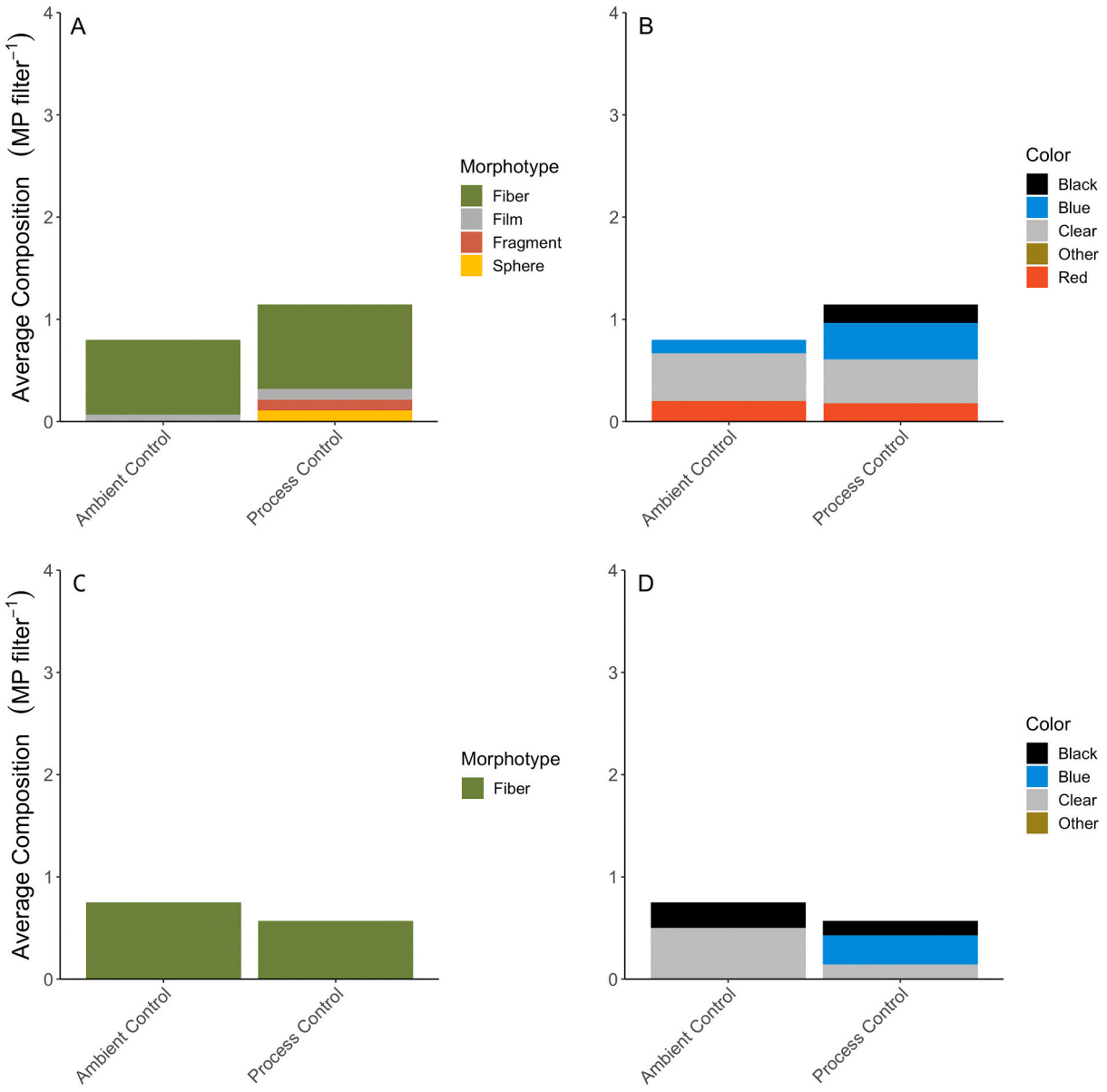

Fig. 5. Composition of average microparticle in control samples for A) regional morphotype, B) regional color composition, C) localized morphotype, and D) localized color composition per filter.

Table 3
Kruskal-Wallis rank sum test on microparticle fiber length including controls for regional and localized sites.

Variable	X^2	Df	p-value
Regional fiber length	12.05	11	0.36
Localized fiber length	8.904	6	0.179

previous studies, and can offer insight into environmental concentrations in the region. Mussels used in regional and local analyses were collected in different years and as a result, were not compared to one another. We acknowledge that the number of mussels collected from each location (10) and the quantity of MP found in each mussel (0–6) were both low, giving little power to the statistical analyses and subsequent lack of differences between sites.

Localized Tatoosh Island mussels were generally uncontaminated; however, the authors note that Tatoosh Island was littered with macro (large) plastic pollution, undetectable in mussels. Chemical analyses revealed that mussels ingested plastic fragments across the Salish Sea: 20 % of ingested MP from the region were synthetic, and most MP with a plastic identification were fragments (5 of 7). We hypothesize the drivers behind high quantities of macro trash on shore are different than consistently high levels of MP in the water. Tatoosh Island experiences winter storm events that likely beach macro trash while usual tidal flushing brings cleaner ocean water and decreases MP encounter rate of mussels.

Mussel MPs analyzed for chemical composition were 20 % plastic. Given the percentage of chemically identified MP in mussels, it is estimated that 0–0.74 MP g^{-1} wet mussel tissue were plastics. We did not chemically identify MP from controls and will therefore discuss chemical identification of mussel particles only. Mussels contained both higher MP concentrations as well as higher proportions of plastic than oysters in the Salish Sea (Martinelli et al., 2020). Mussel MP concentration ranged 0–3.7 MP g^{-1} wet tissue where 20 % were identified as synthetic material while oyster MP ranged 0.02–0.14 MP g^{-1} wet tissue where 2.6 % were identified as synthetic material (Martinelli et al., 2020), a ten-fold difference in contamination between suspension feeding shellfish

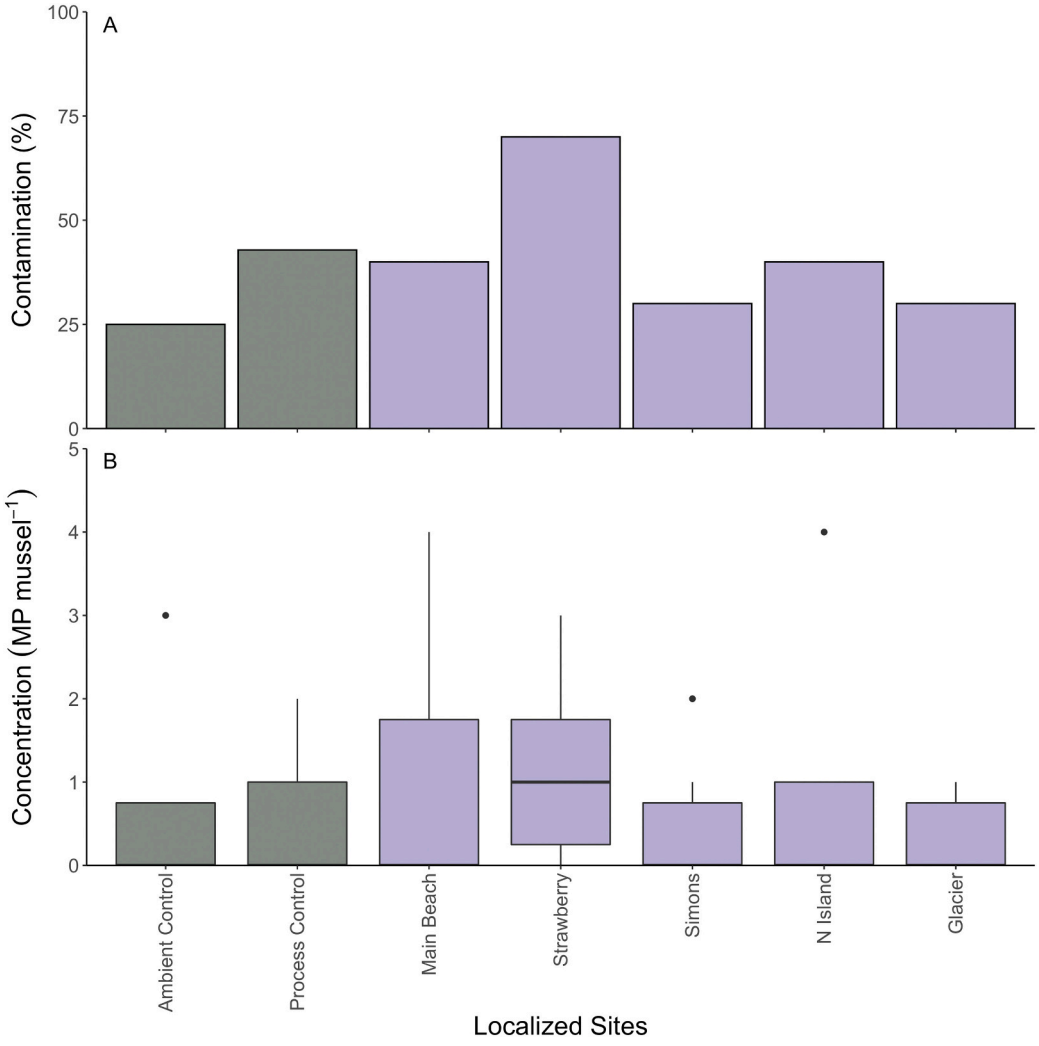

Fig. 6. Localized Tatoosh Island mussel microparticle A) contamination (containing ≥1 MP) percentage and B) concentration (MP mussel^{-1}). Ambient and process controls are on the left in grey. Boxes represent upper and lower quartiles, dots represent outliers, and solid lines within boxes represent median values.

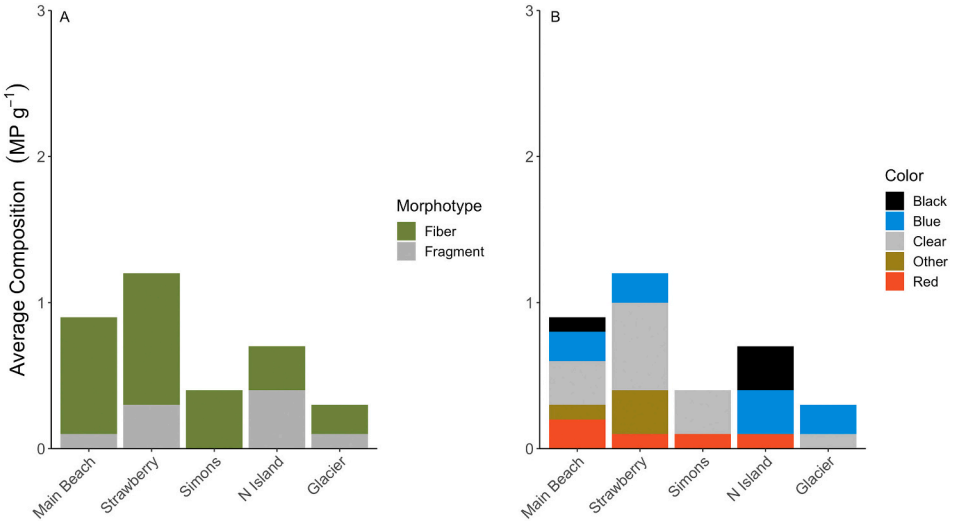

Fig. 7. The average composition of localized Tatoosh Island microparticle A) morphotype and B) color composition per gram of wet mussel tissue (MP g^{-1}) across sites.

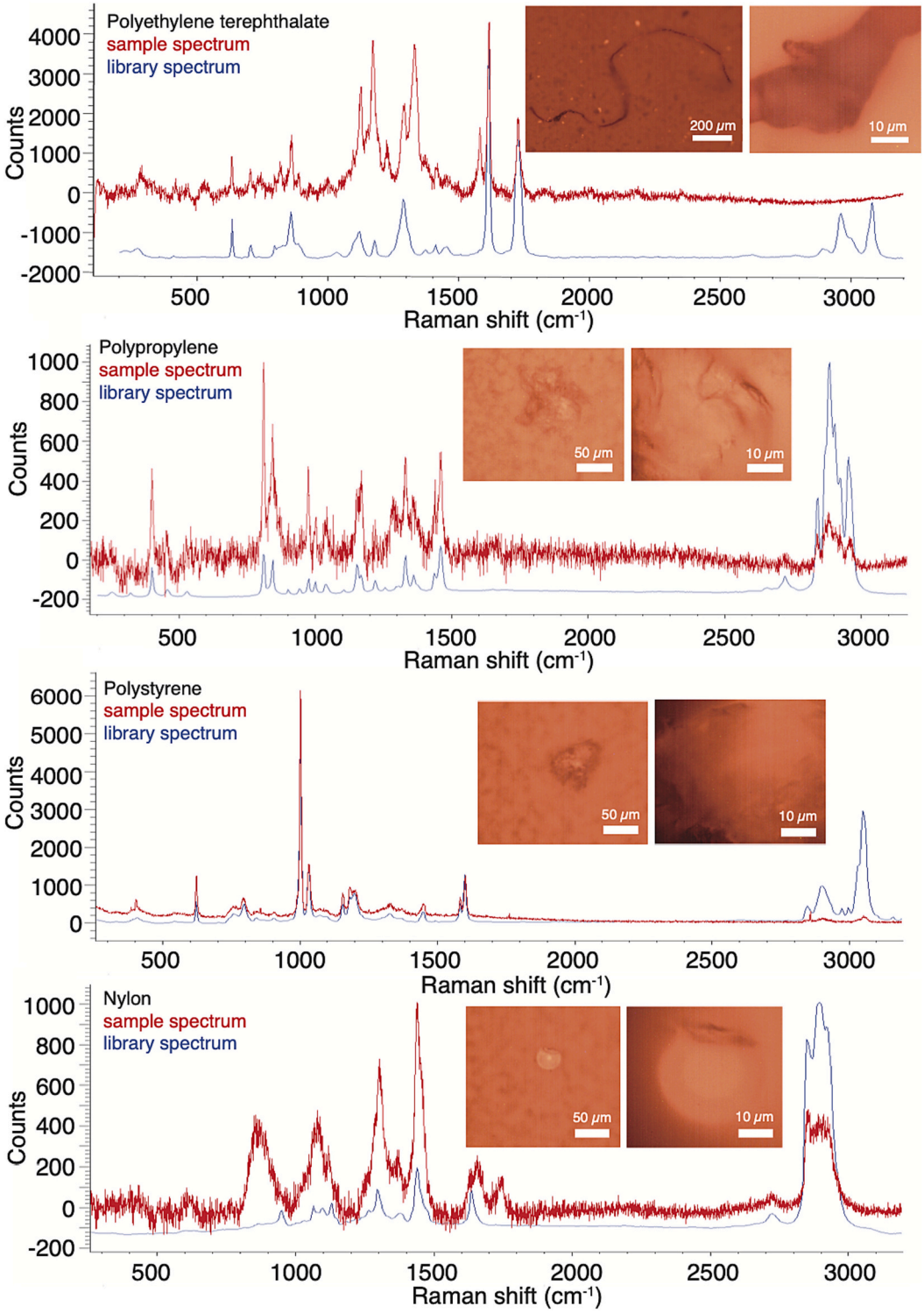

Fig. 8. Raman spectra for microplastics identified in mussel samples.

species. In addition to higher concentrations and percentages of synthetic particles, mussels were found to have more types of synthetic particles, including PET, PP, PS, and nylon (oyster samples consisted of PE, PP, and PS).

We can estimate mussel MP encounter rate by multiplying MP concentrations from water at depth at 0.64 MP L^{-1} (and thus bioavailable to mussels; Harris et al., 2021b) by the clearance rate of mussels at ~1 L h^{-1} (Harris and Carrington, 2019). This calculation estimates a mussel encounter rate of 15.4 MP day^{-1}. If we then multiply this value by mussels' particle processing time (filtration and ingestion) of 18–84 h on average (Kinjo et al., 2019), we would expect mussels to contain 11.5–53.7 MP $mussel^{-1}$, a quantity far higher than what we found (0–3.7 MP $mussel^{-1}$).

Differences in shellfish habitat and feeding mechanics in addition to the physical characteristics of MP offer possible explanations for the difference in concentrations and compositions observed. From a biological perspective, mussels and oysters are known to inhabit different marine environments, where mussels are typically in the rocky intertidal

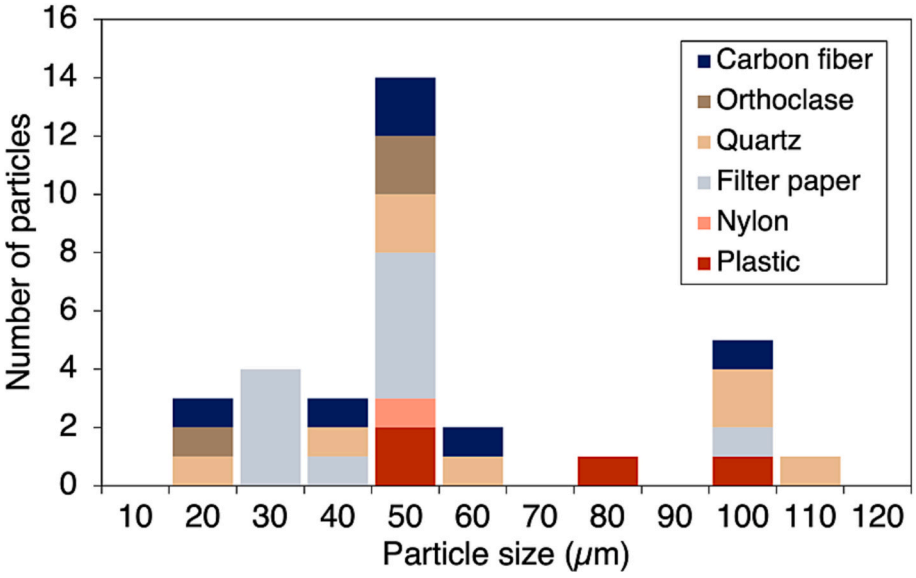

Fig. 9. Size distribution and composition of microparticles identified using Raman microspectroscopy. Synthetic materials consist of three types of plastics, polyethylene terephthalate (PET), polypropylene (PP), polystyrene (PS), as well as nylon. Note that two plastics larger than 800 μm were omitted for clarity.

or attached to firm substrate (e.g., dock, rope) while oysters occupy soft-sediment intertidal zones. Here, we collected mussels from docks or the rocky intertidal, where they likely filtered and ingested particles from near surface waters, which may explain the similarity between mussel and water concentrations previously measured. Further, oysters are exposed to high sediment concentrations and are efficient suspension feeders, able to remove up to 50 % of particles (Ward and Shumway, 2004), perhaps explaining why Martinelli et al. (2020) reported lower MP concentrations in oysters.

Notably, polyethylene (PE) has been identified in Pacific seawater, Salish Sea seawater, and Salish Sea oyster studies (Morét-Ferguson et al., 2010; Covernton et al., 2019; Martinelli et al., 2020; Harris et al., 2021b); however, MP analyzed from mussels in this study did not contain PE. This result is interesting considering that PE is one of the most widely used commodity plastics (Plastic - The Facts, 2022; Morét-Ferguson et al., 2010). Nylon was found in the Salish Sea mussel samples as well as in Pacific oysters near coastal British Columbia, Canada (Covernton et al., 2019), but was not found in Pacific Oysters in the South Salish Sea (Martinelli et al., 2020).

Quartz and orthoclase minerals were also found in mussels. Additional minerals such as calcite were found in previous studies, likely due to high river input in the region (Ripken et al., 2021). In Washington, mussels from five sites, Friday Harbor, Port Townsend, Mukilteo, Kingston, and Browns Point - Tacoma contained minerals mistaken as MP during visual identification. These sites were not concentrated in one basin or geographical area inferring that the mussels contained quartz and orthoclase from sediment input from multiple rivers feeding into the Salish Sea (Fig. 1).

From a physical perspective, characteristics of MP are known to dictate fate and transportation in marine systems (Zhang, 2017). Examining the material properties of MP found in mussels (e.g. density in g ml^{-1}) may offer an explanation of mussel MP concentrations (morphotype, polymer type, and quantities). The most common commodity plastics, PE (e.g. plastic film, thick plastic bottles), PP (e.g. straws, bottle caps, diapers), and PS (e.g. takeout containers, insulation), have similar densities as seawater (1.025 g ml^{-1}) while PET (e.g. beverage bottles, clothing, rope) and nylon (e.g. fabric, food packaging, filaments) are denser than seawater (Table 4). Mussels in this study were collected from docks or rocks relatively near the sea surface, so it is likely that PE is available due to its low density, high buoyancy, and relatively high production. Conversely, quartz and orthoclase minerals are denser than seawater and likely to settle out of the water column (Kooi et al., 2017).

Table 4
Densities of common plastic and minerals found in Salish Sea mussels as well as comparable microplastic studies.

Microparticle Identity	Density (g ml^{-1})	Reference
Polyethylene (PE)	0.857–0.975	https://www.ptonline.com/articles/density-molecular-weight-in-polyethylene
Polypropylene (PP)	0.895–0.92	https://www.plasticseurope.org/en/about-plastics/what-are-plastics/large-family/polyolefins
Polystyrene (PS)	1.05–1.06	https://polymerdatabase.com/polymers/polystyrene.html
Nylon	1.13–1.41	https://www.bpf.co.uk/plastipedia/polymers/polyamides.aspx
Polyethylene terephthalate (PET)	1.29–1.40	(Andrady, 2017)
Carbon fiber	1.75–2.00	https://link.springer.com/content/pdf/10.1007/s11837-005-0217-8.pdf
Orthoclase	2.56	http://webmineral.com/data/Orthoclase.shtml#.YLlQkDZKjUI
Quartz	2.65–2.66	https://www.mindat.org/min-3337.html

Evidence of sediment in visual identification and lack of PE contamination may highlight a selection bias, where mussels are highly capable of selecting MP and egestion mechanisms are too rapid to accurately show MP water concentrations (Dimitrijevic, 2011; Ward et al., 2019). Previous laboratory experiments show that mussel MP ingestion is biased towards small particles and fibers, and that most MP are rejected or quickly egested resulting in significantly lower MP water concentration estimates (Dimitrijevic, 2011, Ward et al., 2019). Our results align with Dmitrijevic (2011) and Ward et al. (2019), where mussel MP contamination is likely lower than expected due to their highly selective filtration abilities.

Another possible reason for the difference in confirmed types of particles between this study and previous research in the Pacific Ocean is that biofouling on PE causes an increase in density, thus bypassing mussel filter zones and landing in benthic substrate available for oysters. Morét-Ferguson et al. (2010) found that low-density polymers such as PE were found to have higher densities when collected from beaches than pristine counterparts, concluding that the increase in density

resulted from biofouling at sea. There is still little research on the mechanisms of biofouling and how that contributes to the distribution of microplastics in the water column.

Microparticle studies performed using mussels may estimate lower MP concentrations than those that actually exist in the water column (Harris et al., 2021b). In this study, mussels were (when possible) collected from marinas which are areas of high anthropogenic activity, and therefore thought to be areas of high MP concentrations. Even so, only one site sampled in the Salish Sea had mussel MP concentrations significantly higher than control samples. Neither water nor biotic sampling methods may reflect the average Salish Sea MP concentration levels. Further, mussel MP concentrations and morphologies in the Salish Sea did not differ by basin or were correlated to water residence times (Supplemental Fig. 1), contrary to previous studies, where areas with high surface basin residence times correlated positively with higher plastic load in surface waters (Mahoney, 2017).

Differences in MP sources, heterogeneous transport of MP, and distinct shellfish feeding mechanisms may contribute to plastic contamination patterns in the Salish Sea. These factors need to be examined separately and in factorial analyses to begin to untangle Salish Sea MP contamination. While mussels may not be the best bioindicator for detecting MP contamination, mussels are ubiquitous, important to human consumption, already used globally to monitor other marine contaminants, accessible, and the ingestion mechanisms are understood (Li et al., 2019; Ward et al., 2019; Ding et al., 2021).

CRediT authorship contribution statement

Lyda S.T. Harris: Ideation, conceptualization, resources, funding acquisition, methodology, investigation, data curation, writing–original draft, writing–review and editing, and supervision. S. Phan: Methodology, investigation, data curation, writing–original draft, writing–review and editing. D. DiMarco: Writing–original draft, writing–review and editing. J. Padilla-Gamiño: Resources and editing. C. Luscombe: Resources and editing. E. Carrington: Resources, funding acquisition, investigation, data curation, writing–original draft, writing–review and editing, and supervision.

Declaration of competing interest

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.

Data availability

Data will be made available through Dryad.

Acknowledgements

Tatoosh Island sites are on Makah Land and sampling was made possible by working with the Makah Ocean Council and Ray Colby. We deeply appreciate the opportunity to work on Makah land and sea and recognize this work was conducted on ceded and unceded lands and waterways of the Coast Salish peoples. This research was supported by the WRF Hall Endowment for Graduate Student Excellence in Biology and the Edmonson Award from the UW Dept of Biology to L. Harris. Part of this work was conducted at the Molecular Analysis Facility which is a part of the National Nanotechnology Coordinated Infrastructure (NNCI), a National Science Foundation-funded effort to coordinate nanoscale research and development activities across the United States, and is supported by NNCI-2025489 and NNCI-1542101. We thank Julieta Martinelli for her guidance, methods, and patience in the lab. We would also like to thank Louise Sutters and Claire Hutchinson for their help measuring and categorizing particles as well as members of the Carrington Laboratory group, Molly Roberts, Matt George, and Hilary Hayford for helpful discussions and brainstorms throughout the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marpolbul.2023.115609.

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