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# Microplastics in Pacific Northwest Bivalves: Ecological Prevalence, Harvester-Consumer Exposure, and Aquarium Exhibit Outcomes

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Microplastics in Pacific Northwest Bivalves: Ecological Prevalence,  
Harvester-Consumer Exposure, and Aquarium Exhibit Outcomes

by

Britta Rahel Baechler

A dissertation submitted in partial fulfillment of the  
requirements for the degree of

Doctor of Philosophy  
in  
Earth, Environment and Society

Dissertation Committee:  
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2020

## Abstract

Up to 23 million metric tons of mismanaged plastics enter the global ocean annually. Microplastics (MPs; <5mm) are small plastic particles generated either intentionally during manufacture or as a result of larger macroplastics (>5mm) degrading. MPs leak into the environment and now pervade every corner of the earth, with implications for animal, plant, ecosystem and human health. It is important to understand localized environmental MP prevalence, distribution and organismal uptake in order to determine the extent of MP pollution, and ultimately unlock solution sets to reduce transmission of MPs into the natural world. In Chapter 2, I quantified MP types, concentrations, anatomical burdens, geographic distribution, and temporal differences in Pacific oysters (*Crassostrea gigas*) and Pacific razor clams (*Siliqua patula*) from 15 coastal sites in Oregon (OR). Both of these species are integral to culture, economy, gastronomy and food security along the United States West Coast. In Chapter 3, I quantified MPs in Pacific razor clams collected from 8 Olympic Coast, Washington (WA) sites, administered questionnaires to recreational razor clammers and subsequently estimated annual MP exposure of those Olympic Coast razor clam harvester-consumers surveyed. In Chapter 4, I designed and installed an educational ocean plastic exhibit at the OR Coast Aquarium. I evaluated time spent in the exhibit, number of times panels were read or interactive elements were touched, determined current and intended single-use plastic reduction and plastic stewardship actions by visitor demographic, gauged

visitor learning after the exhibit experience and recorded suggestions for exhibit improvement.

MPs were present in Pacific oysters and razor clams from all 23 (15 OR and 8 WA) sites studied in Chapters 2 and 3. Among all clams and oysters studied in both OR and WA, over 99% of MPs encountered were fibers. In OR bivalves, whole oysters and razor clams contained an average of  $10.95 \pm 0.77$  and  $8.84 \pm 0.45$  MP pieces per individual, or  $0.35 \pm 0.04$  and  $0.16 \pm 0.02$  MP g<sup>-1</sup> tissue, respectively. Spring-collected oysters contained more MPs than summer-collected oysters. In WA razor clams, average MP burden for whole clams was  $6.75 \pm 0.60$  MP/individual and for cleaned clams (rinsed with guts and gills removed) was  $3.44 \pm 0.25$  MP/individual.

The number of razor clams consumed per meal by WA razor clam harvesters was  $4.27 \pm 0.27$ , which varied by gender and ethnicity, but not income or age. Harvesters ate 0–209 meals/year of razor clams, and 88.3% of respondents fully cleaned razor clams before consuming them. Annual suspected MP exposure for razor clam harvester-consumers was 60–3,070 pieces for cleaned and 120–6,020 for whole clams.

Within our OR Coast Aquarium ocean plastic exhibit, children (0–17 years) touched the three interactive podia more than adults (18–80+ years) and adults read informational panels more frequently than children. Adult visitors increased their desire to help address the ocean plastic problem after interaction with our exhibit and reported willingness to change one or more of the single-use plastic or plastic stewardship actions, with nearly 40% intending to increase use of reusable straws and contribute to cleaning the beach. Of all age groups (18–29, 30–39, 40–49, 50–59, 60–69, 70–79), 18–29 year



olds had the highest change in desire to help address the ocean plastic problem before and after seeing the exhibit, but the lowest likelihood of engaging in single-use plastic reduction or plastic stewardship actions, highlighting a need for targeted outreach to this specific age group. The totality of all three research chapters within this dissertation serve as important references within the growing portfolio of Pacific Northwest MP investigations, and can inform future education, outreach and policy efforts to attenuate environmental MPs and reduce household-level single-use plastic waste generation in the region.

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## Preface

This dissertation is written in the format of individual manuscripts for Chapters 2, 3, and 4; as such, appendices, acknowledgements, references and supplementary materials corresponding to each Chapter can be found at the end of that Chapter's text. Chapter 2 was an invited paper to the *Limnology & Oceanography Letters* Special Issue titled 'Microplastics in marine and freshwater organisms: Presence and potential effects', and was published in February 2020 at doi:[10.1002/lol2.10124](https://doi.org/10.1002/lol2.10124). Chapter 2 data are available in the PDXScholar data repository at <https://doi.org/10.15760/esm-data.1>.

At the time of dissertation submission, Chapter 3 was accepted for publication in *Frontiers in Marine Science: Marine Pollution* (Oct 23, 2020), and Chapter 4 was submitted for publication to *Environmental Education Research* (October 6, 2020). Some repetition of abstract and introductory information may be present due to the manuscript-style format of dissertation Chapters.

## Chapter 1: Introduction

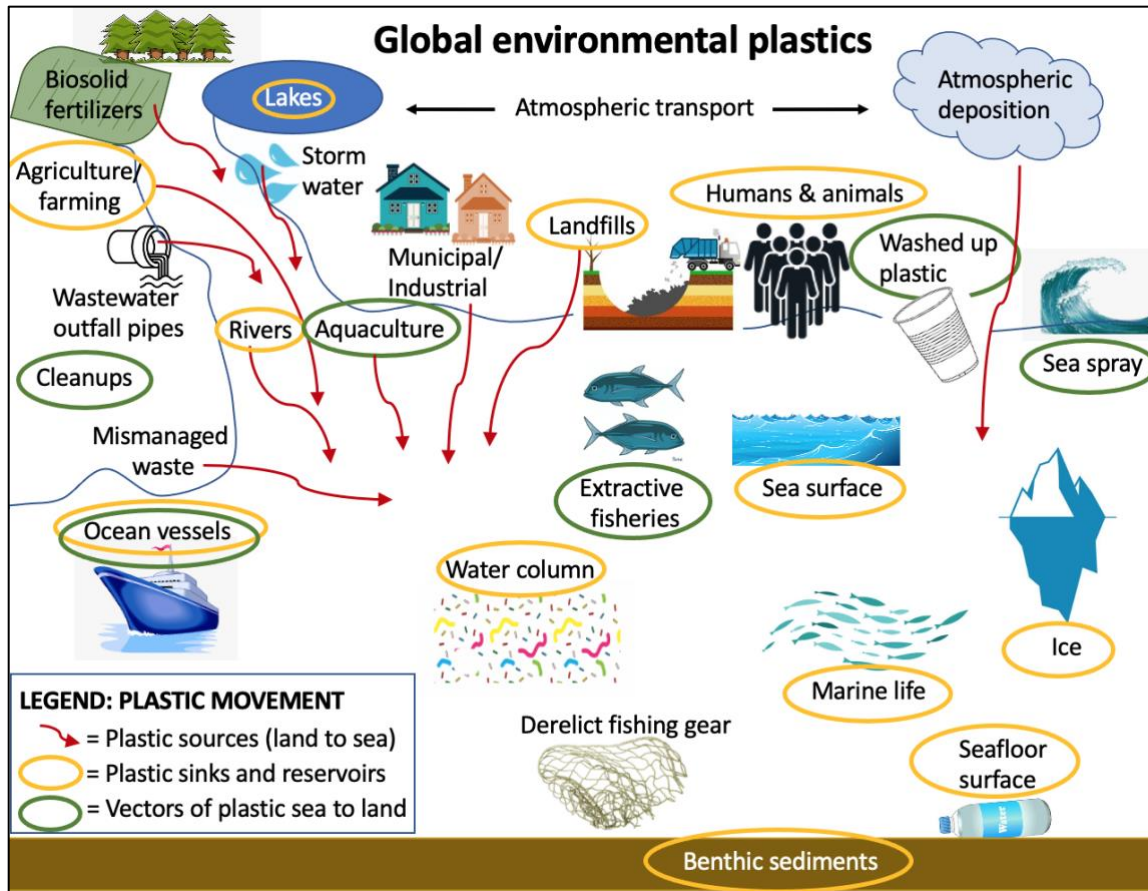
Plastics are synthetic materials comprised of a series of monomers which together make polymers (Thompson et al. 2009). The first fully synthetic plastic was developed in the early 1900's and was coined Bakelite (Crespy et al. 2008). Large-scale plastic production began in earnest in the mid-twentieth century and has increased exponentially since (Gu and Ozbakkalogu 2016), from an estimated annual output of 2 million metric tons per year in 1950 to 380 million metric tons per year in 2015 (Geyer et al. 2017). Plastics now have many important uses and benefits and have become integrated into every facet of modern life and the human experience; as such, sharper increases in plastic production are expected and may eclipse 500 million metric tons per year by 2050 (Geyer et al. 2017; Lau et al. 2020; Napper and Thompson 2020). Unfortunately, widespread reliance on plastics and failure of governments to effectively regulate production and recycling of most plastic products have led to a high propensity for mismanagement. In 2015, 4.9 billion metric tons of plastics filled landfills and polluted the environment globally; by 2050 that number could climb to over 12 billion metric tons per year (Geyer et al. 2017).

Macroplastics, widely defined as plastics greater than 5mm in any linear dimension, break down in the environment when subjected to heat, light, and environmental forces such as wind, wave, UV, biological and mechanical disturbances, into microplastics (MPs; <5mm) and nanoplastics (NP;  $\leq 0.1 \mu\text{m}$ ). MPs cannot be categorized as a single contaminant; instead, they represent a diverse suite of

contaminants due to their highly variable chemical compositions, shapes, colors, and forms (Rochman et al. 2019). MPs are categorized as either primary (polymers intentionally manufactured to be <5mm in size) or secondary (polymers degraded from larger plastics, resulting in particles <5mm in size) particles, and exist as fragments, pellets, filaments, films, fibers or fiber bundles (Rochman et al. 2019). It is estimated that up to 23 million metric tons of mismanaged macroplastics and 1.5 million metric tons of MP unceremoniously enter the global ocean each year (Borrelle et al. 2020; Boucher et al. 2017), impacting more than 700 marine species through direct ingestion of polymers or entanglement in derelict debris (Gall and Thompson, 2015).

MPs are ubiquitous and found in terrestrial, aquatic and marine ecosystems (Figure 1). They are present in air, soil, fresh, brackish, and salt water, sea ice, glacial ice, flora and fauna, as well as numerous other environmental media (e.g. Cole et al. 2011; Rochman et al. 2015; Datu et al. 2019; Wright et al. 2020; Guo et al. 2020). These contaminants largely originate from land-based sources and frequently make their way into the aquatic and marine environment. Atmospheric deposition of MPs has been observed in urbanized areas as well as presumed pristine areas like alpine glaciers, suggesting that wind and particles in the atmosphere can carry plastics to every corner of the earth (Rochman and Hoellein 2020). Microfibers have been associated with atmospheric deposition and much higher rates of fallout occur during periods of rain, suggesting precipitation is able to ‘push’ airborne plastics back down to the ground (Horton et al. 2017). These once-airborne particles are transported by stormwater to nearby waterways, and out to the ocean.





**FIGURE 1.** Transmission of plastics into the environment: Examples of sources and routes of plastic transmission from terrestrial to aquatic environments, environmental sinks and reservoirs, and vectors of plastic transport from aquatic to terrestrial realm.

Rivers transport up to 2.4 million tons of plastic into global oceans each year, carrying nearly 30,000 tons to the sea annually by North and Central American rivers alone (Lebreton et al. 2017). Sewage sludge applied to agricultural fields as fertilizer is a significant source of microplastics in the environment, with nearly half of the 5.9 million metric tons of microfibers generated through laundering since the 1950's applied through this method (Gavigan et al. 2020). Microplastics subsequently become air and waterborne and are able to travel long distances. Plastic-based mulches, polytunnels, and polymer-coated seeds used in agriculture are also sources of plastics that can eventually break

down and become mobile in the environment (Horton et al. 2017). Stormwater runoff and wastewater treatment plant (WWTP) effluent deliver significant volumes of MPs to coasts and oceans as well. Microfibers comprise the greatest proportion of MPs in the coastal environment, and are derived from wastewater, gray water and stormwater inputs, among other sources (Figure 1). Considering a single load of synthetic clothing can generate up to 700,000 microfibers which are flushed through gray water into WWTP (Napper and Thompson 2016), a single city or urban area could easily generate hundreds of billions of fibers per day even if wastewater is heavily filtered.

MP transmittance to the marine environment has been increasing for decades in a trend similar to global plastic production (Ostle et al. 2019). As MP abundance in oceans increase, the propensity of those particles to become bioavailable to marine organisms also increases (Auta et al. 2017). Once waterborne, MPs are mistakenly ingested by marine and aquatic organisms. While the physical properties of MPs are worrying due to observed incorporation of the particles into their guts, gills, and tissues, the chemical properties are perhaps even more concerning. During plastic production, one or multiple fillers, plasticizers, pigments, antimicrobials, heat or UV stabilizers, or flame retardants are used (Rochman et al. 2019). There are several thousand distinct chemical additives used in plastics to make them flexible, resistant to heat, or durable. These additives can constitute the majority (over 70%) of a plastic product's total weight, though on average represent about 4% (Smith et al. 2018). Many additives incorporated into plastics are associated with well-established environmental and human health risks. More than 50% of plastics are composed of materials known to be hazardous to environmental or human

health (Rochman et al. 2019). These additives can become mobilized from the polymer matrix and leach into the surrounding environment. Phthalates, Bisphenol-A (BPA), polybrominated diphenyl ethers (PBDE), nonylphenols (NP), and antioxidants are the most commonly encountered plastic additives detected in the ocean (Hermabessiere et al. 2017). Some of these compounds have been widely studied and are known endocrine disruptors. BPA, for example, is a xenoestrogen which is often incorporated into plastics or used to line food and beverage containers. When ingested even at low doses, BPA interacts with important hormone receptors. Although it degrades quickly in the environment, it persists due to continual inputs from landfill leachate, environmental plastics and other sources (Flint et al. 2012). As plastics degrade, the surface area to volume ratio increases, exposing even more chemical additives and increasing their propensity to leach. Another feature of MPs is their incredible ability to sorb contaminants from the surrounding environment. Due to the hydrophobicity, chemical and molecular properties of plastics, they are extremely effective at collecting and harboring concentrations of waterborne chemical contaminants up to 1 million times more concentrated than in the surrounding waters (Mato et al. 2001). Contaminants known to be associated with microplastics include heavy metals, pesticides, dioxins and furans, flame retardants, and pharmaceutical and personal care products (Avio et al. 2015).

The transfer of plastics and plastic additives into marine organisms and up trophic levels have been demonstrated in the laboratory and found in field-collected samples (de Sá et al. 2018). After ingestion, organisms either eliminate MPs through excretion or

pseudofeces, translocate the particles throughout the body, or retain them in one or more organs (Auta et al. 2017). Retained particles may be transferred to higher trophic levels within food webs (Farrell and Nelson 2013; Nelms et al. 2018).

The presence of potentially harmful additives and ability of contaminants to sorb to plastics consumed by animals are some of the chief concerns attributed to MP ingestion, as multiple deleterious physiological effects have been demonstrated when organisms are exposed to MPs or MP-associated chemicals (e.g., Avio et al. 2015, Hermabessiere et al. 2017, Guzzetti et al. 2018, Prokić et al. 2019). Documented impacts include neurotoxicity, oxidative stress, impaired growth and behavior, decreased reproductive output, increased immune response, decreased feeding, weight loss, energy depletion, and damage to cells and DNA (Lusher et al. 2017).

In concert, MPs, their associated additives and sorbed contaminants threaten the health of marine organisms, the environment, and potentially human seafood consumers as well (Carbery et al. 2018; Barboza et al. 2018). We now know that, as apex predators and consumers of seafood in the food chain, humans are subsequently exposed to MPs. Recent studies have estimated humans are exposed to significant amounts of MPs not only through food and beverages consumed such as seafood, beer, honey, and salt, but also from drinking water and breathing air (e.g., Catarino et al. 2018; Cox et al. 2019; Zhang et al. 2020). Plastic-associated chemicals such as BPA show troubling correlations with chronic human illnesses such as cardiovascular disease and type 2 diabetes in humans (Smith et al. 2018). While humans are likely able to clear the vast majority (>90%) of ingested MPs from their systems (Smith et al. 2018), questions still remain

regarding chemical transfer from MPs to the body, and the fate of plastics that are not excreted. Similar to animals, individual susceptibility and harm to human consumers from MP consumption is likely variable due to exposure route, personal traits, health, or other existing stressors. The potential cumulative effects of chronic exposure to MPs and associated chemicals in humans have not been well-documented and are therefore critical avenues of future research.

Bivalves have been used as bioindicators for evaluating the health of aquatic and marine environments since the 1960s, when mussels and clams were first used to monitor environmental pesticides and heavy metals (Farrington et al. 2016). Since then, entire long-term contaminant monitoring programs have been developed using bivalves as bioindicators. As filter feeders, bivalves are particularly vulnerable to contaminants in the estuarine and open coast environments they inhabit. Environmental MP concentrations in sediments and water often have been correlated with MP burdens in coastal bivalves (e.g., Mathalon and Hill 2014; Qu et al. 2018; Su et al. 2018). Numerous elements make bivalves ideal sentinel organisms for biomonitoring of environmental contaminants, and there are many benefits to using bivalves to monitor environmental contaminants like MPs. First, they are widely distributed around the globe in both freshwater and marine habitats. Their filter-feeding food acquisition strategy and fixed location in the environment (usually on the sea floor or in freshwater sediments) mean the pollutant levels found within represent a specific geographic locale. They can accumulate chemicals in the water up to several orders of magnitude higher than the surrounding waters, making them extremely useful for identifying and monitoring environmental

pollutants. Because sample contamination due to air and waterborne particles are problematic in the field of MPs research, bivalves may be superior study organisms compared to other aquatic species because of their hard shell enclosures. These can effectively seal out contamination until opened in the laboratory, and also make organisms fairly robust for transportation purposes. Lastly, many species of clams, oysters and mussels are consumed by humans (often whole) and comprise key markets in aquaculture and wild fisheries. A recent study by Su et al. (2018) identified the Asian clam (an invasive species found all around the world) as an excellent bioindicator of MP pollution in freshwater ecosystems, whereby clam tissue MP concentrations and sizes were strongly related to MP concentrations and sizes in surrounding benthic sediments. This may be due to the fact that this species in particular combines filter-feeding and deposit-feeding strategies. A review by Li et al. (2019) found a positive correlation between MPs in mussel tissue and in environmental water samples worldwide.

While there are advantages to studying MPs in bivalves as indicators of environmental MP pollution, there are also disadvantages. For example, not all plastic types are necessarily bioavailable to bivalves. While it has been demonstrated that plastics less dense than seawater (such as expanded polystyrene, low density polyethylene and polypropylene) as well as those denser than seawater (polyvinyl chloride, high density polyethylene) are somewhat mixed in the water column, they are not equally distributed so monitoring of bivalve tissues may not provide a full picture of the environmental plastic material types in a particular geographic area. Some bivalves are also able to preferentially select for food particles of certain size fractions, and may

therefore expel MPs encountered on their gills or labial palps in pseudofeces rather than ingesting them (Li et al. 2019). This preferential sorting could be problematic if attempting to extrapolate MP concentrations in bivalves to environmental concentrations.

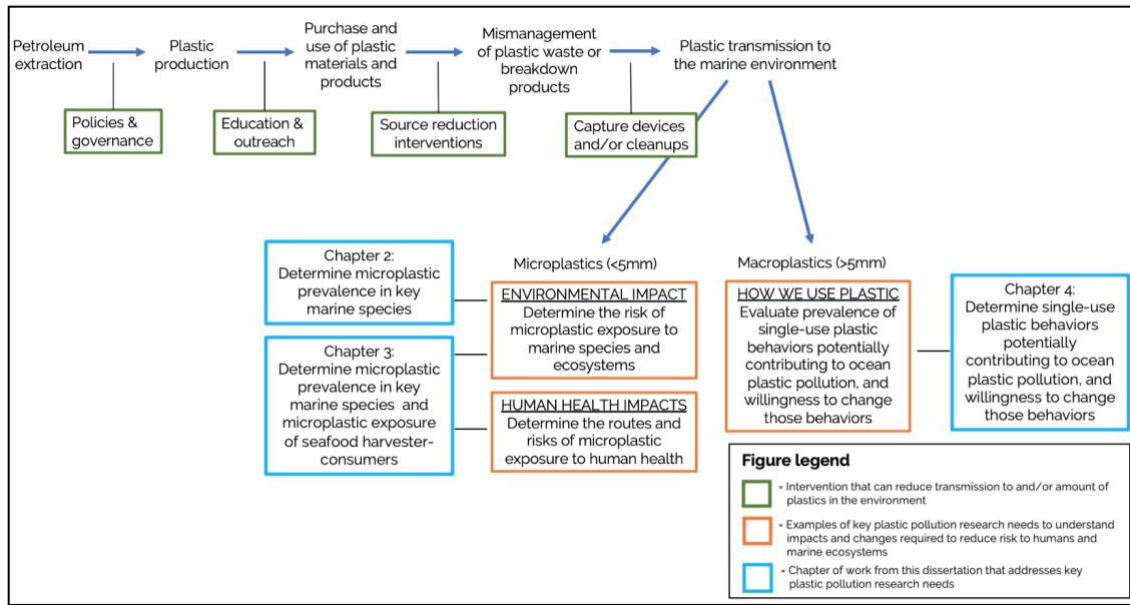
The Pacific Northwest (PNW) coast supports diverse marine species, which have nourished, sustained and inspired local peoples since time immemorial. Present-day species targeted in commercial, tribal usual and accustomed, sport, and recreational fisheries contribute greatly to local culture, spirituality, economies, gastronomy, and food security (Olson 1936; Charles et al. 2004; Anderson and Parker 2009; Lepofsky et al. 2015). Pacific oysters and Pacific razor clams were selected as target species for investigation of MPs in my research due to their feeding mode, limited movement, importance to PNW economy and culture, and representativeness of both estuaries and open coast sandy beach environments characteristic of large swaths of the Oregon (OR) and Washington (WA) coasts. The field of MP research in marine and coastal environments is rapidly expanding and evolving, yet relatively little is known about the prevalence of MPs in many commercially-important species, particularly in North America (Baechler et al. 2020a). It is important to understand localized environmental MP prevalence, distribution and organismal uptake in order to determine the extent of MP pollution in a particular geography. Although MPs have been identified in sand samples collected from PNW beaches (Horn et al. 2020) and in water from its rivers (Valine et al. 2020), knowledge of MP burdens in OR and WA seafood species remain limited (e.g., Baechler et al. 2020b, Martinelli et al. 2020).

This dissertation spans the broad themes of human plastic uses and behaviors, plastic prevalence in the environment, and shellfish as a vector of MPs to human seafood consumers. I apply these themes by identifying plastic uses and environmental stewardship actions that could be targeted in future education efforts, outreach endeavors and policies (Chapter 4), investigating MP pollution in PNW coastal bivalves (Chapters 2 and 3), and quantifying MP exposure by bivalve harvester-consumers (Chapter 3). The specific goals of my research are to determine:

1. Spatial, temporal and anatomical variation in MP burdens in Pacific oysters and Pacific razor clams on the OR coast (Chapter 2);
2. MP burdens in Olympic Coast, WA razor clams, and subsequent annual MP exposure by razor clam harvester-consumers (Chapter 3); and
3. Educational gains and behavioral changes of visitors at the OR Coast Aquarium after interaction with an ocean plastic exhibit (Chapter 4).

My research aims to address ocean plastic pollution research needs including: 1) prevalence of MPs found in marine species, which can ultimately help determine risk of MPs to those species; 2) human exposure to MPs through consumption of seafood items, which can ultimately inform human health risk impacts of MP exposure; and 3) prevalence of certain single-use plastic behaviors and willingness to change those behaviors, which can ultimately help reduce mismanaged plastics and plastic pollution in the ocean (Figure 2).





**FIGURE 2.** Plastic transmission to the environment: Process, interventions, examples of ocean plastic pollution research needs, and dissertation chapters that address one or more of the research needs presented.

Chapter 2 is the first study to document MPs in any OR marine species, and is also the first to determine MP burdens in Pacific razor clams anywhere in the world. It provides important coast-wide data to compare MP burden across species, seasons, and sites. Chapter 3 builds upon Chapter 2 by expanding the geographic area of known Pacific razor clam MP burdens, first confirming MP presence and spatial variability in razor clams on the Olympic Coast, WA, and then estimating annual harvester-consumer MP exposure from razor clam consumption. Chapter 4 leverages insights gained in the previous two chapters by approaching plastic pollution solutions through education and behavioral changes of aquarium visitors, as individual consumer choices, behaviors, and habits are contributors to the global plastic economy, driving demand, use and fate of those products and their packaging. Chapter 5 provides a synthesis of findings, highlighting how this work improves our understanding of MP prevalence in PNW

seafood species and suggests the work's utility towards advancing future ocean plastic pollution education, outreach and policies aimed at reducing plastic pollution in the region.

In total, this work is critical to document prevalence of MP contaminants in PNW bivalves valued for their importance to coastal ecology and human consumers. It provides a crucial reference to measure future change of MPs in coastal organisms and allows comparisons to be drawn between the Olympic Peninsula, WA and OR coasts. It contextualizes human harvester-consumer MP exposure from one key PNW seafood species relative to other known exposure routes (e.g., inhalation of air, ingestion of drinking water). This research helps identify environmental MP characteristics, providing important clues regarding potential MP sources that could guide mitigation, engineering, and policy development. It can help inform key interventions to reduce the transmission of macro and MPs into the environment (Figure 2), and provide resource practitioners, seafood industry members, tribal members, seafood consumers, the scientific community, educators and the general public with information to reference in future management, education and outreach initiatives, and MP attenuation efforts.

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## Chapter 2: Microplastic Concentrations in Two Oregon Bivalve Species– Spatial, Temporal, and Species Variability

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### 2.1 Introduction:

Microplastics, plastics 0.0001-5 mm in any linear direction (UNEP 2016), are found in nearly every environment on earth (Thompson et al. 2004). These tiny fragments, pellets, filaments, and fibers originate from both marine and land-based sources, infiltrating aquatic ecosystems worldwide through pollution, runoff, wastewater, and atmospheric deposition (Zhang 2017). Globally, the overwhelming number of single-use and non-degradable plastic items has led to widespread microplastic pollution. Plastics are manufactured to be durable, so degradation can take hundreds to thousands of years, posing a pervasive and severe problem for ecosystems as well as a human health concern (Cole et al. 2011; Wang et al. 2016).

While spatial distributions of microplastics in the environment are highly complex, areas with high human population, coastal recreation and tourism pressures generally yield high environmental microplastics (Barnes et al. 2009; Hantoro et al. 2019). Microplastics represent a diverse set of contaminants which encompass infinite combinations of plastic densities, sizes, shapes, surface textures, and chemical properties

(Rochman et al. 2019). Once transmitted into the environment, microplastics are subjected to an array of dynamic hydrological, biological, and atmospheric processes including surface currents, tides, biofouling, mechanical and ultraviolet degradation, precipitation, storm events, and more. While human presence may correlate with microplastic prevalence, it is unclear what specific environmental processes best predict fate and transport of these pernicious particles (Zhang et al. 2017). Density has been thought to ultimately determine environmental fate, with denser plastics like polyvinyl chloride (PVC) and polyethylene terephthalate (PET) settling to the benthos and low density polymers such as polystyrene (PS), polypropylene (PP) and polyethylene (PE) remaining in surface waters; however, a recent review of global surface water and sediment data indicate a mixture of high and low density microplastics in water and sediment samples, attributed to influences of varied environmental and biological processes in coastal areas (Wright et al. 2013; Hantoro et al. 2019).

Rivers have been well-established as vectors of plastics into coastal and marine environments (Zhang 2017). These dynamic waterways transport between 1.2 and 2.4 million tons of plastic into global oceans each year, with up to 28.8 thousand tons transmitted annually through North and Central American rivers alone (Lebreton et al. 2017). A study investigating microplastic concentrations in surface waters from two Los Angeles, California rivers quantified an input of roughly two billion microplastic pieces into coastal waters in the span of just three days (Moore et al. 2005). Expanded to an annual output, these two rivers transport over 240 billion microplastics per year to the California coast. Stormwater runoff and wastewater treatment plant (WWTP) effluent

also contribute significant microplastic burdens to coastal environments (e.g., Napper et al. 2016; Carr et al. 2016; Mintenig et al. 2017). Microfibers, generally broken down from laundered clothing items or from derelict fishing gear, are the most prevalent form of microplastic in the nearshore environment (Barrows et al. 2018; de Falco et al. 2019).

Organisms inhabiting coastal environments are subjected to ambient environmental conditions, including microplastic contamination that may exist in surrounding waters, substrata, or in the air. Aquatic filter- and suspension-feeding organisms can encounter microplastics in the marine or freshwater column, mistake them for food items, and ingest them (Hantoro et al. 2019). This transfer of plastics from the environment into aquatic food webs has been documented across diverse taxonomic groups, life histories, habitats, and feeding types (e.g., Cole et al. 2011; Akpan 2014; Rochman et al. 2015; Waite et al. 2018). After uptake, microplastics can adhere to organs or become incorporated into guts, gills, and tissues of organisms, decreasing energy uptake and impairing muscle function and reproduction (e.g., Von Moos et al. 2012; Sussarellu et al. 2016; Ribeiro et al. 2017; Kolandhasamy et al. 2018). Microplastics may also sorb harmful contaminants that, once ingested or incorporated in tissues, are released into the organism (Teuten et al. 2007, 2009). In some studies, environmental microplastic concentrations have been directly correlated with microplastic burdens in coastal bivalves (Mathalon and Hill 2014; Li et al. 2016; Qu et al. 2018; Hantoro et al. 2019).

As filter feeders, bivalves are particularly vulnerable to contaminants in the estuarine and open coast environments they inhabit. The Pacific Northwest (PNW) region of North America supports an array of filter-feeding shellfish species, which have been

inextricably tied to the natural history and cultural heritage of the area for millennia. Fishery and aquaculture sectors serve as important anchors of the region, with Pacific oysters and razor clams playing particularly significant roles in food security and the economy (Crossett et al. 2013). Pacific oysters (*Crassostrea gigas*) have been commercially farmed in the PNW since introduction of the species in the early 1900's (Glude and Chew 1982). These filter feeders consume particulates in the water column, such as plankton and other organic material, and reach commercial size (100-150 mm, maximum length 250 mm) over 2-4 years (Pauley et al. 1988; Harris 2008). Pacific razor clams (*Siliqua patula*) are native to the PNW and are found on intertidal beaches. They have been harvested by first nations and tribal peoples for centuries, and in state-managed recreational and commercial fisheries since the 1950s. Consuming phytoplankton, razor clams grow rapidly in the first year attaining lengths up to 90 mm, and a maximum length of 16 cm over their 6-year lifespan (Link 2000).

Microplastic concentrations in field-collected Pacific oysters have been documented worldwide facilitating comparisons between samples grown in Oregon versus other regions; however, there is no published literature on microplastic prevalence or effects in Pacific razor clams. We initiated this study to answer the question *What variables predict microplastic concentrations in Oregon Pacific oysters and Pacific razor clams?*

Flowing between the U.S. states of Washington and Oregon is the Columbia river, the largest river on the North American continent with a Pacific Ocean terminus. We predicted the Columbia would be a major vector of microplastics, causing elevated

burdens in our study species at the northernmost study sites and attenuated burdens with increased distance from the river. Coastal tourism is highest during the summer months (May to October). This creates increased use of beaches and waterways for recreation and an uptick in laundering needs, so we hypothesized that concentration of environmental microplastics in waters and coastal organisms would be higher in summer than spring. We hypothesized that gut-tissue would contain more microplastics than non-gut tissue due to retention of microplastics in the gut of bivalves observed in previous studies (e.g., Browne et al. 2008; Ward and Kach 2009; Sussarellu et al. 2016; Woods et al. 2018). Because microplastics may become lodged in gills or other organs (Woods et al. 2018), we predicted a positive relationship between organism size and microplastic burden – that larger individuals would contain more microplastics. We examined these expectations through field-collection of Pacific razor clams and purchase of Pacific oysters at 15 locations, during two seasons, taking biological measurements and investigating whole, gut-tissue, and tissue-only samples.

## **2.2 Methods:**

### *Field sites, sample collection, processing, and microplastic enumeration*

A total of 141 Pacific oysters and 142 Pacific razor clams were collected from 15 sites during low tides in spring (April 27-28) and summer (July 21-31), 2017 (Table 1). Whole oysters were purchased from growers at six grower sites during both seasons. One oyster grower was selected from each of six Pacific oyster-producing bays. In this report, oyster grower names are withheld and are coded randomly as OY1–OY6. Oyster shell



length averaged 125.39 mm (range= 77.67–197.66 mm) and wet tissue weight averaged 30.97 g (range= 8.51–101.67 g; Chapter 2 Appendix 1).

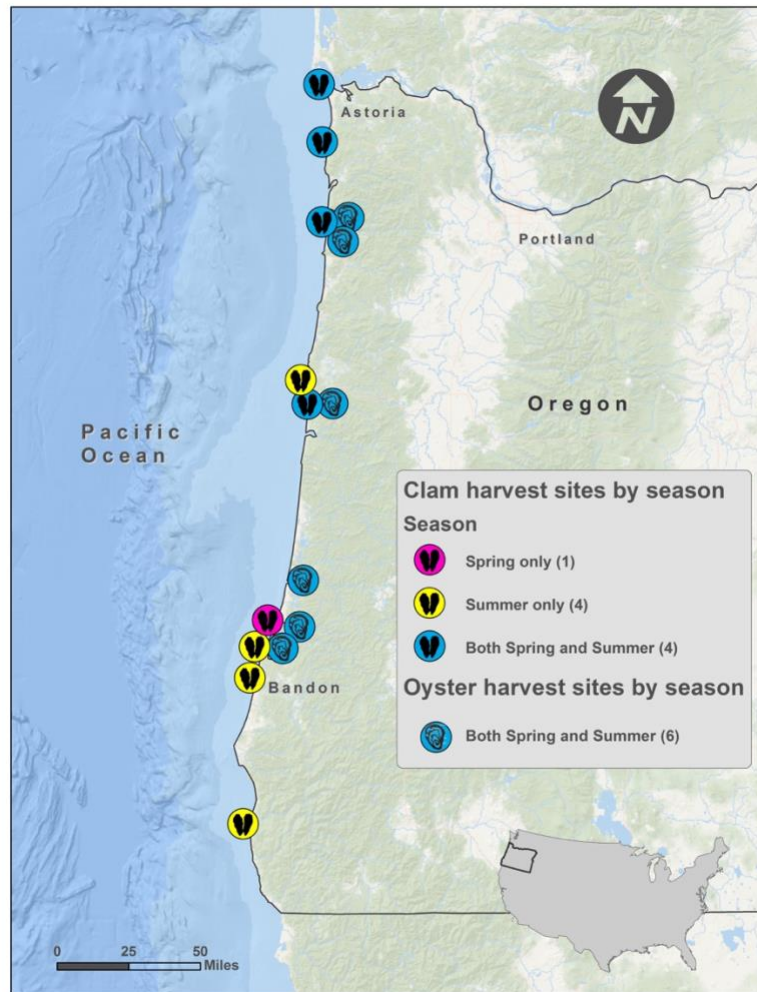
**TABLE 1.** Number of samples analyzed and average microplastic burden in Oregon Pacific oysters and Pacific razor clams by site and season, and average, minimum, and maximum lengths of microplastics identified at each site. Pacific oyster site names are randomized and coded OY1–OY6. Pacific razor clam sites are listed by latitude from north to south.

Species	Site	SPRING				SUMMER				BOTH SEASONS				
		# samples analyzed			Microplastic burden		# samples analyzed			Microplastic burden		Avg MP length in mm (SE) <sup>f</sup>	Min MP length <sub>3</sub> (mm) <sup>d</sup>	Max MP length <sub>3</sub> (mm) <sup>e</sup>
		Whole organisms	Gut tissue	Non-gut tissue	Avg # MP/sample (SE) <sup>a</sup>	Avg # MP/g tissue (SE) <sup>b</sup>	Whole organisms	Gut tissue	Non-gut tissue	Avg # MP/sample (SE) <sup>a</sup>	Avg # MP/g tissue (SE) <sup>b</sup>			
Pacific oyster	OY1	10	0	0	13.60 (2.60)	0.55 (0.34)	10	3	3	9.6 (2.56)	0.49 (0.17)	1.10 (0.05)	0.16	5.37
	OY2	12	0	0	10.33 (1.92)	0.35 (0.29)	11	3	3	6.81 (1.58)	0.21 (0.05)	1.32 (0.06)	0.18	5.85
	OY3	10	0	0	14.60 (3.53)	0.62 (0.49)	10	2	3	8.5 (2.13)	0.28 (0.07)	1.24 (0.07)	0.12	6.08
	OY4	10	0	0	17.50 (3.85)	0.39 (0.28)	10	3	3	5.20 (1.54)	0.10 (0.02)	1.23 (0.05)	0.11	5.42
	OY5	10	0	0	16.30 (2.80)	0.85 (0.41)	10	3	2	7.7 (1.48)	0.57 (0.16)	1.24 (0.05)	0.10	5.56
	OY6	10	0	0	10.80 (2.01)	0.31 (0.16)	11	3	2	11.00 (3.03)	0.50 (0.17)	1.31 (0.07)	0.15	5.40
Pacific razor clam	Clatsop Beach	10	0	0	9.50 (1.21)	0.18 (0.09)	10	3	3	7.60 (1.01)	0.13 (0.02)	1.30 (0.07)	0.19	5.04
	Cannon Beach	5	0	0	10.00 (1.48)	0.18 (0.05)	9	3	3	9.78 (1.47)	0.17 (0.02)	1.43 (0.09)	0.18	8.19
	Cape Meares	10	0	0	8.00 (2.82)	0.19 (0.22)	7	3	3	7.00 (2.57)	0.62 (0.33)	1.19 (0.07)	0.16	4.27
	Agate Beach	0	0	0	N/A	N/A	10	3	3	6.3 (0.91)	0.09 (0.01)	1.32 (0.12)	0.26	5.73
	Newport S. Beach	13	0	0	9.69 (1.49)	0.21 (0.12)	10	3	3	9.30 (0.84)	0.14 (0.02)	1.44 (0.07)	0.21	7.04
	Coos Bay	12	0	0	10.50 (1.55)	0.23 (0.10)	0	0	0	N/A	N/A	1.46 (0.08)	0.31	4.73
	Bastendorff Beach	0	0	0	N/A	N/A	5	0	0	14.80 (1.24)	0.25 (0.01)	1.38 (0.10)	0.27	6.09
	Whiskey Creek	0	0	0	N/A	N/A	10	3	3	6.30 (0.87)	0.11 (0.02)	1.54 (0.13)	0.36	5.71
	Gold Beach	0	0	0	N/A	N/A	10	3	3	8.70 (1.47)	0.12 (0.02)	1.29 (0.09)	0.26	4.93

Notes: Avg. = Average; MP = Microplastic; SE = ± Standard Error; # = Number; mm = millimeter; <sup>a</sup> Average number of MP per sample (SE); <sup>b</sup> Average number of MP per gram of tissue (SE); <sup>c</sup> Average MP length in mm (SE); <sup>d</sup> Minimum MP length at site in mm (SE); <sup>e</sup> Minimum MP length at site in mm (SE); OY1-OY6: Oyster site (randomized). Reported values include background and processing fiber levels.

Razor clams were collected from nine sandy beach sites stretching from Clatsop in the north, to Gold beach, near the California border, in the south (Figure 1). Of the nine clam sites, four were sampled in both spring and summer, providing a temporal snapshot of microplastic frequencies. Collection was performed in coordination with Oregon Department of Fish and Wildlife (ODFW) and Oregon Department of Agriculture (ODA), which greatly augmented collection efforts to achieve desired sample size. Clam sites were selected based on ODFW knowledge of existing clam populations, feasibility of sample collection (access, tides, clam shows), and with a goal of sampling a

large swath of the coast. Summer clam sampling was more robust than spring because it corresponded with a coast-wide ODFW survey and coincided with lower tides than spring. Razor clam shell length averaged 113.89 mm (range = 56.00–132.52 mm) and wet tissue weight averaged 55.71 g (range= 5.84–92.11 g; Appendix A: Chapter 2).



**FIGURE 1.** The 2017 sample collection sites along the Oregon coast delineated for Pacific oysters and Pacific razor clams [Map credit: K. Scully-Engelmeyer; Service Layer Credits: Esri, Garmin, GEBCO, NOAA, NGDC, and other contributors; Sources: Esri, USGS, NOAA].

All samples were transported on ice to the Applied Coastal Ecology laboratory at Portland State University (PSU) in Portland, Oregon, in clean 2 L glass Mason jars. Shell

and tissue measurements were collected with a digital Mitutoyo caliper and Ohaus balance accurate to 0.01 mm and 0.01 g, respectively. Bivalve shells were rinsed with DI water to remove sand, mud, and debris, were shucked into clean 120 mL Mason jars and frozen at -20°C.

Samples were thawed and digested for 24 h in a laminar flow fume hood using 10% potassium hydroxide (KOH). Digestion began with the first organism from each site and season, then proceeded to the second organism from each site, until all samples were processed. Samples were poured through a 7.6 cm diameter, 63 µm stainless steel sieve. Material retained on the sieve was rinsed into clean, labeled glass petri dishes. Petri dishes with Petristickers® affixed to the bases were placed in a drying oven at 40° C for 24 h and stored in sealed tubs prior to microscope processing. Due to high levels of organic material and sand granules remaining in clam samples after initial digestion, a second 10% KOH digestion combined with hypersaline density separation (330g/L Fisher Chemical Certified ACS Crystalline NaCl) was utilized. Samples were analyzed under a Leica M165C stereomicroscope (10-120x magnification) connected via a Leica IC80 HD camera to a computer running Leica Application Suite X imaging software. Each suspected microplastic encountered was measured and particle type (fiber, fragment, film, foam, bead, unknown), color, and maximum length were recorded. To determine material type for microplastics, a subset of identified fibers was randomly selected using random number generation to determine: 1) sample dish, then 2) segment of each dish (segment numbers 1-16) from which to extract 26 suspected microplastics. The first fiber visually encountered in the randomly generated dish and segment was selected for

validation. Fibers were analyzed using a Thermo Nicolet iS10 Fourier Transform Infrared Spectrometer (FTIR) equipped with an Attenuated Total Reflectance accessory at the University of New Hampshire Instrumentation Center. Spectra for each microfiber were acquired using 256 to 1024 scans depending on size and width. Automatic software comparison of microfiber spectra to a set of Thermo Nicolet Omnic™ FTIR spectral libraries was used to generate a best match.

#### *Gut/tissue separation*

During summer sampling, three individual organisms from each site (with the exceptions of Bastendorff Beach and Coos Bay) underwent a separation of digestive organs from other tissues. For Pacific oysters, gut-tissue samples included the visceral mass, esophagus, diverticular gland, midgut, and stomach. In razor clams, gut-tissue samples included the stomach, small intestine, and crystalline style. All remaining tissue was classified as non-gut tissue. Separated gut- and non-gut tissues underwent the same digestion and microscope analyses as whole organism samples.

#### *Quality control: Contamination quantification and prevention*

One hundred percent cotton clothing, cotton lab coats, and nitrile gloves were worn at all times during sample processing, digestion, and analysis procedures. All shucking implements and glassware were rinsed three times with deionized (DI) water filtered to 0.22 µm. To quantify procedural contamination, 11 replicates of 50 mL filtered DI water were frozen in 4 oz jars and underwent the same digestion and analysis process

as organism samples. One procedural blank per week was chemically digested alongside field samples on a randomly generated day. Additionally, three procedural blanks were collected to quantify contamination introduced by the secondary digestion and hypersaline density separation of razor clam samples.

During microscope analysis, a petri dish containing filtered DI water was placed adjacent to each sample on the microscope base and left open to the air to quantify airborne contaminants. After sample analysis, the control petri dish was analyzed for microplastics; any particles detected were assumed to be contamination and were measured and categorized.

#### *Data analysis and availability*

To identify differences between sample sites, seasons, and anatomical burdens, analysis of variance (ANOVA) and Welch's t-tests were conducted in the R statistical program (v1.2.1335) using the aov and t.test functions (R Core Team 2019). Linear regression models were used to examine relationships between biological parameters (shell length, body weight) and microplastic burdens. Microplastic concentrations are expressed as number of microplastics per sample or mean number of particles  $\text{g}^{-1}$  tissue (wet weight; whole organisms only). Number of microplastics per sample and number of microplastics per gram of tissue variables were log transformed ( $\log x+1$ ) prior to statistical analysis. The statistical cutoff (alpha) for all tests was 0.05 with Standard Error (SE) reported. Data and metadata are available in the Portland State University PDXScholar data repository.

## 2.3 Results:

### *Quality control*

Numerous measures were taken to minimize procedural contamination, but as with other studies (e.g., Li et al. 2015; Davidson and Dudas 2016; Qu et al. 2018; Su et al. 2018) it was not completely eliminated. Contamination in procedural controls ( $4.91 \pm 1.11$ ), microscope blanks ( $0.20 \pm 0.03$ ), and, for razor clams, a secondary digestion and separation step ( $1.0 \pm 0.0$ ) was quantified (Appendix B: Chapter 2). From these controls and procedural blanks, total contamination in oyster and clam samples was estimated at 5.11 and 6.11 microplastics per sample, respectively. Average microplastic length detected as contamination (n= 124) for all sample types was  $1.67 \pm 0.11$ mm, and most frequently detected colors in blanks and controls were colorless (79%) and blue (10%). As with multiple other studies (e.g., Li et al. 2015; Davidson and Dudas 2016; Li et al. 2016; Li et al. 2018; Qu et al 2018; Su et al. 2018; Rochman et al. 2019), we report microplastics detected in blank samples (Appendix B: Chapter 2), rather than performing a blank-subtraction on environmental results since controls were intended to provide a range of possible contamination levels introduced through laboratory procedures. As such, our reported numbers are estimated maximum possible microplastic concentrations.

### *Microplastic occurrence in study species*

A total of 3,053 suspected microplastics were isolated from 320 whole-organism, gut-tissue, and non-gut tissue samples. Over 99% of particles were microfibers (n= 3,026) averaging 1.34mm in length (range= 0.10–8.72 mm). The remaining <1% of

microplastics were categorized as fragments (n= 12), beads (n= 5), films (n= 5), foams (n= 2), or unknown (n= 3). Colorless, blue, gray, and black were the most commonly observed fiber colors at 62%, 21%, 7% and 4%, respectively.

Microplastics were present in organisms at all sites during both sampling periods and across the entire geographic range sampled with some discernible patterns (Table 1, Figure 2). Mean microplastic concentrations in whole organisms was  $10.95 \pm 0.77$  in Pacific oysters (range= 0–42) and  $8.84 \pm 0.45$  in razor clams (range= 0–38). Mean microplastic burden per gram of tissue in whole organisms was significantly different between oysters ( $0.35 \pm 0.04 \text{ g}^{-1}$  tissue) and razor clams ( $0.16 \pm 0.02 \text{ g}^{-1}$  tissue;  $t = -6.43$ ,  $df = 199$ ;  $p = <0.0001$ ), but number of microplastics per whole organism was not significantly different (Table 2;  $t = -1.16$ ,  $df = 235$ ,  $p = 0.25$ ). FTIR analysis of 26 individual fibers extracted from whole organisms indicates material types of polyethylene terephthalate (n=8), acrylic (n=2), aramid (n=1), zein (n=1) and cellophane, a cellulose-based material (n=10). Because cellophane exhibited a low spectral match percentage (20-67%) relative to other materials (aramid: 68%; all others: 80-95%), we believe the cellophane-characterized fibers should be more broadly deemed cellulose-based material types. Additional fibers (n=4) were run but no material type matches were determined, most likely due to small fiber width and concomitant low signal to noise data.

**TABLE 2.** Microplastic burden of Oregon Pacific oysters and Pacific razor clams (in number of microplastics per whole individual and per gram of whole organism tissue). Significant differences are indicated in **bold**.

Species	Microplastic burden comparison	# Microplastics per gram of tissue				# Microplastics per whole organism			
		Test type	Test statistic	Degrees of freedom (df)	Significance	Test type	Test statistic	Degrees of freedom (df)	Significance
Both species (Pacific oyster and Pacific razor clam)	Whole oyster to whole razor clam (both spring and summer)	T-test	T = -6.43	df = 199	<b>p = &lt;0.0001</b>	T-test	T = -1.16	df = 235	p = 0.25
	Whole oyster to whole razor clam (spring only)	T-test	T = -6.21	df = 89	<b>p = &lt;0.0001</b>	T-test	T = -2.63	df = 103	<b>p = &lt;0.001</b>
	Whole oyster to whole razor clam (summer only)	T-test	T = -3.24	df = 103	<b>p = 0.002</b>	T-test	T = -1.29	df = 112	p = 0.19
Pacific oyster	Gut vs tissue	N/A				T-test	T = 0.48	df = 31	p = 0.63
	Spring vs summer	T-test	T = 2.57	df = 121	<b>p = 0.01</b>	T-test	T = 4.41	df = 121	<b>p = &lt;0.0001</b>
Pacific razor clam	Gut vs tissue	N/A				T-test	T = -0.55	df = 39	p = 0.59
	Spring vs summer	T-test	T = -0.29	df = 50	p = 0.77	T-test	T = 0.09	df = 71	p = 0.93

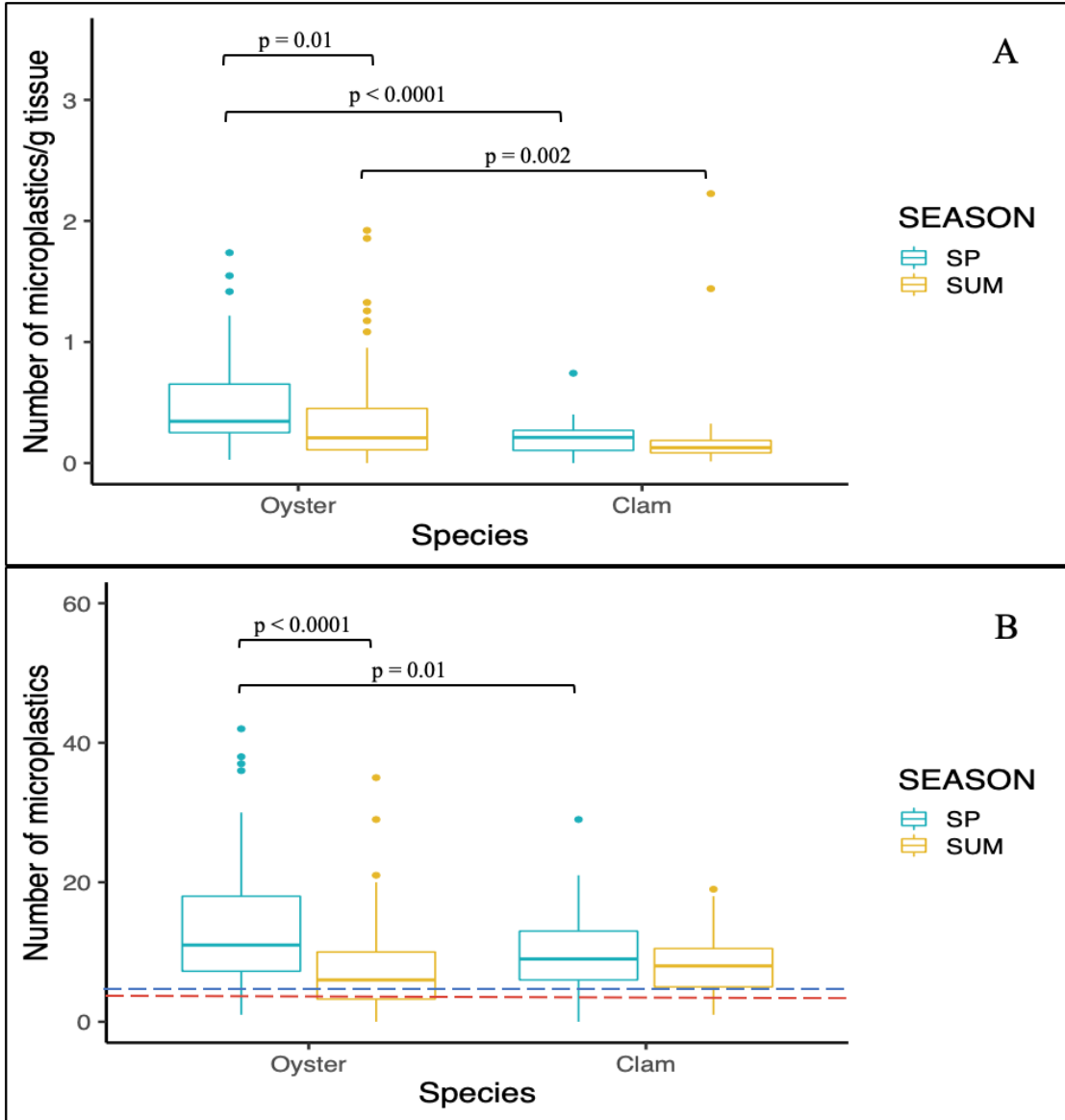
*Notes:* Number of microplastics per gram of tissue was not compared for gut and tissue samples, as mass was recorded for whole organism samples only. T-tests were conducted on log transformed data. To test temporal difference in Pacific oysters, data from all six sites sampled in both spring and summer were compared; for razor clams, only the four sites sampled in both seasons (Clatsop Beach, Cannon Beach, Cape Meares, Newport South Beach) were compared.

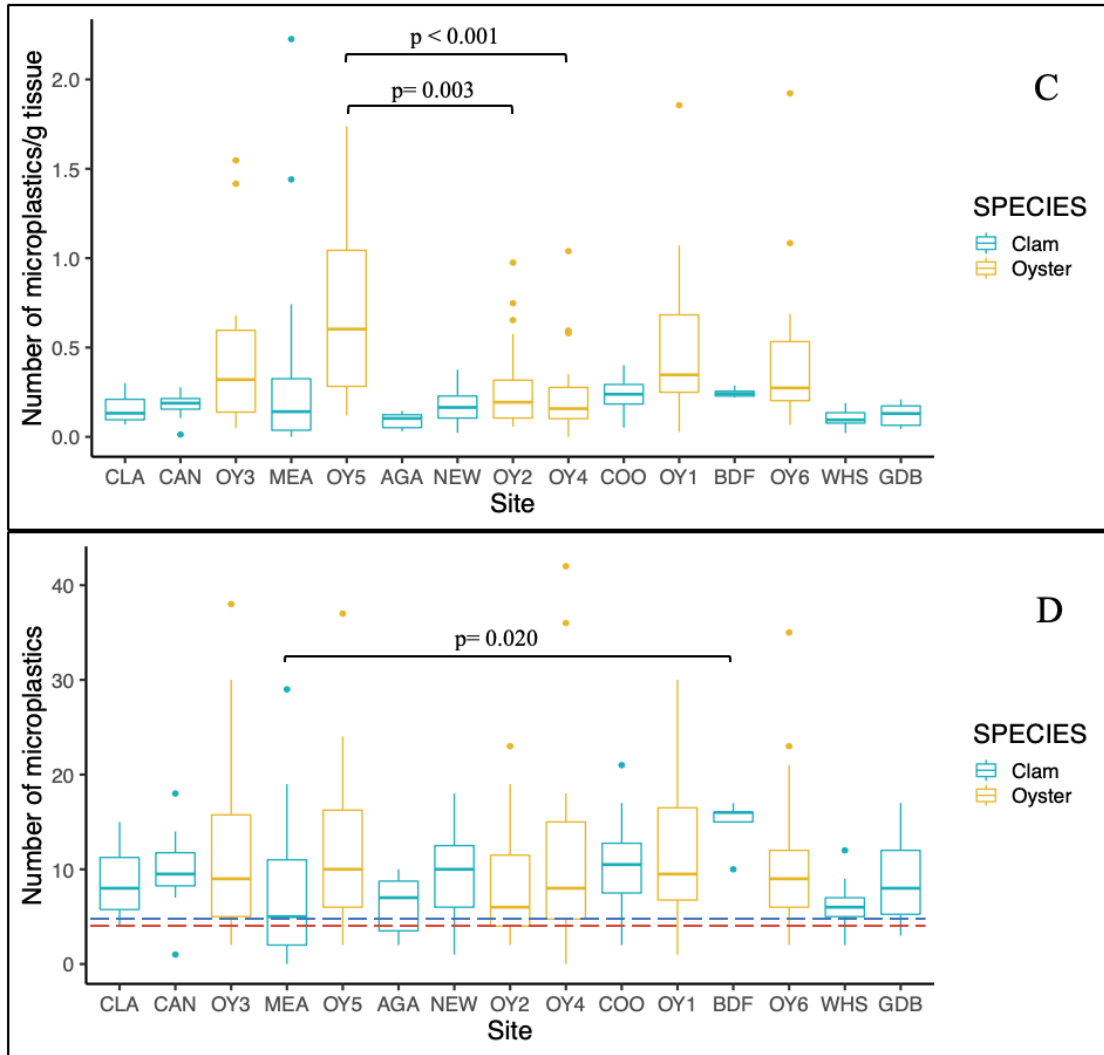
### *Temporal differences*

Significant intra- and inter-species differences in microplastic burdens were detected during the two sampling periods (Figure 2A and B). Spring Pacific oysters contained significantly more microplastics than summer; on average, whole spring oysters contained  $13.74 \pm 1.16$  microplastics ( $0.45 \pm 0.05 \text{ g}^{-1}$  tissue) whereas summer oysters contained  $8.16 \pm 0.88$  ( $0.26 \pm 0.05 \text{ g}^{-1}$  tissue; whole organism:  $t = 4.41$ ;  $df = 121$ ;  $p < 0.0001$ ; MP  $\text{g}^{-1}$  tissue:  $t = 2.57$ ;  $df = 121$ ;  $p = 0.01$ ). There was no significant temporal difference in microplastic burden for clams when the four sites sampled in spring and summer were compared (Clatsop Beach, Cannon Beach, Cape Meares, Newport South Beach). Spring razor clams contained  $9.54 \pm 0.81$  microplastics per whole individual ( $0.19 \pm 0.02 \text{ g}^{-1}$  tissue) whereas summer had  $8.35 \pm 0.51$  ( $0.14 \pm 0.04 \text{ g}^{-1}$



tissue; whole organism:  $t = 0.09$ ;  $df = 71$ ;  $p = 0.93$ ; MP  $g^{-1}$  tissue:  $t = -0.29$ ;  $df = 50$ ;  $p = 0.77$ ). When comparing spring oysters to spring razor clams, spring oysters contained more microplastics  $g^{-1}$  tissue (Table 2;  $t = -6.21$ ;  $df = 89$ ;  $p = <0.0001$ ) and more microplastics per whole sample (Table 2;  $t = -2.63$ ;  $df = 103$ ;  $p = 0.01$ ). Summer oysters contained more microplastics  $g^{-1}$  tissue than summer razor clams (Table 2;  $t = -3.24$ ;  $df = 103$ ;  $p = 0.002$ ), but not more plastics per whole organism (Table 2;  $t = -1.29$ ;  $df = 112$ ;  $p = 0.19$ ).





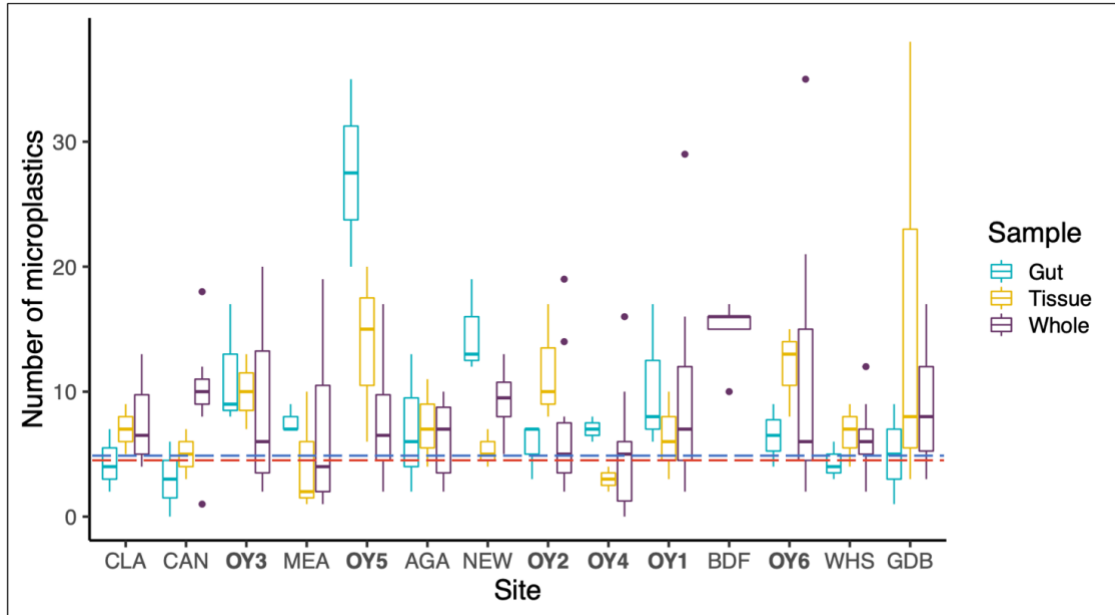
**FIGURE 2.** (A-B): Number of microplastics by season (SP= spring (teal) and SUM= summer (gold)) and species for Oregon Pacific oysters and Pacific razor clams: A) per gram of whole-organism tissue, and B) per whole organism. Welch's T-tests were run to determine seasonal intra- and inter- species differences in log transformed values. P-values show significant differences in microplastic burdens for seasons and/or species pairs indicated. (C-D): Number of microplastics in Oregon Pacific oysters (gold) and razor clams (teal): C) per gram of whole-organism tissue, and D) per whole organism. Dashed blue line indicates average contamination level for razor clams (6.11 microplastics per sample); dashed red line indicates contamination level for oysters (5.11 microplastics per sample). ANOVA and post-hoc Tukey tests were run for each species to determine significance. P-values show significant differences in microplastic burdens for site pairs indicated. Data are combined for both spring and summer sampling periods. Reported values include background and processing fiber levels. Sites are arranged north to south by latitude. OY1-OY6: Randomized oyster sites; CLA= Clatsop Beach; CAN= Cannon Beach; MEA= Cape Meares; AGA= Agate Beach; NEW= Newport; COO= Coos Bay; BDF= Bastendorff Beach; WHS= Whiskey Run; GDB= Gold Beach. Reported values include background and processing fiber levels.

### *Site differences*

ANOVA and post-hoc Tukey tests revealed site-specific differences in microplastic burdens per gram of whole oyster tissue from two site pairings (Figure 2C;  $p < 0.001$  and  $p = 0.003$ ). Site-specific differences in number of microplastics per individual were not detected in oysters (Figure 2D;  $F = 0.56$ ;  $df = 5$ ;  $p = 0.73$ ). For razor clams, site-specific differences in microplastics per gram of tissue were not detected (Figure 2C), but were for microplastics per individual in one site pairing (Figure 2D;  $F = 2.54$ ;  $df = 8$ ;  $p = 0.020$ ).

### *Anatomical burdens*

Microplastics were detected in whole organism, gut-tissue, and non-gut tissue samples in both species from all sites sampled in the summer, except at Bastendorff Beach where sample size precluded separate gut and tissue analyses, and Coos Bay, which was not sampled in summer (Figure 3). Microplastic burden (number of plastics per sample) did not differ between gut-tissue and non-gut tissue in either species (Table 2); Oysters:  $t = 0.48$ ;  $df = 31$ ;  $p = 0.63$ ; Clams:  $t = -0.55$ ;  $df = 39$ ;  $p = 0.59$ ). In oysters, average microplastic burden was  $10.69 \pm 2.01$  in gut-tissue and  $9.41 \pm 1.30$  in non-gut tissue samples. In razor clams, average microplastic burden was  $6.57 \pm 1.02$  in gut-tissue and  $7.43 \pm 1.64$  in non-gut tissue samples.



**FIGURE 3.** Comparison of sample types for summer-collected Pacific oysters (site names bolded) and Pacific razor clams. Reported values include background and processing fiber levels. Gut= gut-tissue, Tissue= non-gut tissue, Whole= whole organism. Dashed blue line indicates average contamination level for razor clams (6.11 microplastics per sample); dashed red line indicates contamination level for oysters (5.11 microplastics per sample). Sites are arranged north to south by latitude. OY1-OY6: Randomized oyster sites; CLA= Clatsop Beach; CAN= Cannon Beach; MEA= Cape Meares; AGA= Agate Beach; NEW= Newport; BDF= Bastendorff Beach; WHS= Whiskey Run; GDB= Gold Beach.

### *Shell length, body weight, and microplastic burden*

Regression analyses revealed shell length (in mm) was not significantly correlated with number of microplastics per whole organism in oysters ( $F= 0.081$ ,  $df=122$ ,  $R^2= -0.008$ ,  $p= 0.777$ ) or razor clams ( $F= 0.421$ ,  $df=118$ ,  $R^2= -0.005$ ,  $p= 0.518$ ). Similarly, body weight (in g) was not significantly correlated with number of microplastics per whole organism in oysters ( $F= 0.430$ ,  $df= 122$ ,  $R^2= -0.005$ ,  $p= 0.514$ ) or razor clams ( $F= 1.355$ ,  $df=118$ ,  $R^2= 0.003$ ,  $p= 0.247$ ).

## 2.4 Discussion:

Microplastics were present in both Pacific oysters and Pacific razor clams collected from all 15 Oregon coast sample sites in both spring and summer 2017. All whole organisms (n= 245) except one oyster and one razor clam contained at least one plastic particle. Microplastic concentrations varied significantly by season in oysters but not razor clams. Limited site-specific differences in microplastic burden were detected for oysters but not razor clams. Contamination in our samples combined with relatively small sample size may have influenced the lack of site differences during statistical analyses. No anatomical microplastic burden differences were detected between gut- and non-gut tissues in either species, and organism size did not correlate with microplastic burden. Both razor clams and Pacific oysters are low trophic level species important to both the ecology of Oregon's nearshore and estuarine environments and humans who culture or consume them. To our knowledge, this is the first study to document microplastics in Pacific razor clams and Pacific oysters harvested in Oregon. Various edible oyster and clam species have been found to contain microplastics elsewhere in the world, including Asia, British Columbia, and Europe (e.g., Mathalon and Hill 2014; Davidson and Dudas 2016; Van Cauwenberghe and Janssen 2014; Li et al. 2015; Su et al. 2018). In this study, the average number of microplastics found in Pacific oysters and razor clams ( $0.35 \pm 0.04 \text{ g}^{-1}$  tissue and  $0.16 \pm 0.02 \text{ g}^{-1}$  tissue) was low compared to average concentrations in Pacific oysters in France, China, and Tunisia of 0.47, 0.62 and 1.5 items  $\text{g}^{-1}$  tissue (Van Cauwenberghe and Janssen 2014; Teng et al. 2019; Abidli et al. 2019), mussels from China of 2.2–2.4 items  $\text{g}^{-1}$  tissue (Li et al. 2016; Li et al 2015), and

manila clams from British Columbia, Canada of 0.9–1.7 items g<sup>-1</sup> tissue (Davidson and Dudas 2016). Concentrations from this study are in the range of those found in blue mussels in France and Belgium of 0.23 and 0.26 items g<sup>-1</sup> tissue (Phuong et al. 2018; de Witte et al. 2014), and are the low end of concentrations found in manila clams in China of 0.3–4.9 items g<sup>-1</sup> tissue (Su et al. 2018). These patterns may be result from the relatively small human population residing on the Oregon coast.

As this is the first study to document prevalence of microplastics in Pacific razor clams (*S. patula*), no comparisons are possible between our Oregon samples and other areas. Due to the importance of the recreational, commercial, and tribal razor clam fisheries in the broader PNW, microplastic burden data from other states and territories in the region should be collected to help elucidate possible larger-scale patterns in prevalence.

In estuarine-grown oysters, collection season appears to influence microplastic burden more than harvest location. Additional research is needed to identify the environmental or anthropogenic factors driving higher microplastic burden in oysters in the spring. Seasonal microplastic differences in oysters but not razor clams may be a function of habitat. Oysters inhabit estuarine environments, which receive land-based stormwater and wastewater inputs before ocean-facing beaches do; therefore, pulse inputs of microplastics may be more concentrated in estuaries than along the open coast. Precipitation was at least 100% higher than normal in all coastal counties and up to three orders of magnitude higher in some coastal areas in April 2017 compared to July 2017, which was characterized by at least 50% lower than normal precipitation in all coastal

counties (NOAA Northwest River Forecast Center 2017). Therefore, seasonal differences in oysters may be driven, in part, by seasonal precipitation and resultant stormwater fluctuations. Another possibility is that the nature of clothing laundered in the spring – cold weather clothes, possibly dominated by insulating synthetic materials - may increase microfiber levels in WWTP outputs when compared to clothing items laundered in the summer. Other potential seasonal factors include temperature-associated influences on metabolic and feeding rates, which may be depressed during colder seasons, and life history events like spawning and associated physiological responses. Differences in aquaculture techniques, such as degree of plastic use by oyster growers, may contribute to variation in oyster microplastic burdens between sites and over time; however, grower-specific culture techniques were not assessed in this study and previous studies in the Pacific Northwest have failed to find a connection between aquaculture and microplastic burden in cultured Pacific oysters and manila clams when compared to wild-grown organisms (Davidson and Dudas 2016; Covernton et al. 2019). Temporal differences identified in this study indicate oysters may be able to clear microplastics from their system over time, as previously shown in laboratory studies where manila clams and blue mussels (29-40mm in length) eliminated microplastics in feces and pseudofeces when depurated in clean water, with up to 60% of particles cleared from the body in as little as 9 h (Xu et al 2017; Woods et al. 2018). However, elimination of microplastics was not detected in blue mussels (50-55mm length) during a depuration period of two hours (Rist et al. 2018). While, in these examples, depuration was studied in bivalves smaller than our study organisms (Appendix A: Chapter 2), the results are promising and warrant



further research. Depuration of oysters or razor clams in freshwater for some period of time prior to sale may be a fruitful avenue for reducing anthropogenic debris in those seafood items.

Visual microscopy is routinely used in microplastics research due to the relatively wide availability of microscopes, but it likely introduces error to microplastic counts. Recent studies indicate visual microscopy can either overestimate or underestimate microplastic counts depending on particle shape and size (Song et al. 2015); thus, additional validation methods should be used to supplement visual analysis methods. In this study, FTIR techniques were used to ground truth material composition of a randomly-selected subset (n=26) of the 2,428 microfibers found in whole samples, which were subsequently identified as polyethylene terephthalate (n=8), acrylic (n=2), aramid (n=1), zein (n=1) and cellophane, a cellulose-based material (n=10). Our low percentage of validated fibers was due to funding limitations and lack of on-site equipment. Polyethylene terephthalate, acrylic, and aramid fibers have been previously found in organisms (e.g., Li et al. 2015; Nelms et al. 2018; Li et al. 2018), and zein (a corn-based protein used in bioplastics) has been isolated from wastewater treatment plant (WWTP) sludge (Bayo et al. 2016). Fibrous cellophane, the putative material type comprising the largest proportion of successfully validated fibers (n=10), is made of heavily modified cellulose but has previously been categorized as a microplastic in studies that identified cellophane fibers in bivalves (Li et al. 2016; Ding et al. 2018; Li et al. 2018). Due to low spectral match percentage of cellophane (20-67%) relative to other materials matched to

known spectra (aramid: 68%; all others: 80-95%), we believe the cellophane-characterized fibers should be more broadly deemed cellulose-based material types.

Our lower size limit of detection for microplastics was 0.063 mm owing to the mesh size of the sieve used, so microplastics smaller than 0.063 mm in length may be underestimated using these methods. Microplastics between 0.10–8.72 mm in length were included in this report, as they are of equal interest as microplastics fitting the conventional 0.0001–5 mm definition. Future studies on these and other bivalve species should include methods capable of detecting both micro and nanoplastics ( $1 \times 10^{-6}$ – $1 \times 10^{-4}$  mm), as particles between  $1 \times 10^{-4}$  and  $2 \times 10^{-2}$  mm can penetrate internal organ barriers (Lusher et al. 2017).

In this study we found that all whole organisms (n= 245) except one oyster and one razor clam contained at least one microplastic, though we acknowledge that some of these detections may have been influenced by contamination in the laboratory. For this reason, we ran several types of blank and control samples during processing and analysis to quantify it. Microfiber contamination may have been due to presence in KOH pellets used for chemical digestion, fibers shed from clothing, laboratory furniture (chairs), or airborne particles. Average contamination represented 46.7% of the average microplastic burdens reported for whole oysters, and 69.1% of average microplastic reported for whole clams. While contamination in this study appears high, it is consistent with similar studies that report between 51 and 94% of detected microplastic values in mussels and clams may represent contamination (Mathalon and Hill 2014; Davidson and Dudas

2016). Contamination documented in this and other microplastic investigations highlights the ubiquity of anthropogenic microfibers in the environment.

The degree to which microplastics pose a threat to coastal marine ecology or bivalve predators (including humans), is still unclear; however, this study provides valuable insights about spatial and temporal variability in microplastic prevalence in important commercial species, sheds light on potential ecological concerns related to microplastic contamination, and serves as a baseline from which future microplastic studies in the region can draw comparisons. Future research on extent of microplastic encounter rates, consumption, and effects on biological endpoints are critical to better understand potential population-level effects on bivalves and marine organisms around the world.

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### **Chapter 3: Microplastic Exposure by Razor Clam Recreational Harvester-Consumers Along a Sparsely Populated Coastline**

*Special note:* This article was first submitted to the journal *Frontiers in Marine Science: Marine Pollution* in August 2020 and was accepted for publication on Oct 23, 2020.

#### **3.1 Introduction:**

Coastal organisms are subjected to a myriad of environmental stressors ranging from the predictable, such as changing tides, predation (e.g., by sea otters, fish, birds and whales), and seasonal food availability, to the less predictable including storm events, disease, toxins, and environmental contaminants. Though numerous definitions exist, microplastics (MPs) – polymers between 1  $\mu\text{m}$  and 5 mm in size (Frias and Nash 2019)– represent a diverse contaminant suite (Rochman et al. 2019). These particles, either manufactured at this size or formed as breakdown products of larger plastic items, make their way to coastal environments via household gray water, wastewater treatment plant effluent, stormwater, rivers, atmospheric deposition and other means (e.g., Napper et al. 2016; Lebreton et al. 2017; Mintenig et al. 2017; Zhang 2017; Zhang et al. 2020a; Zhang et al. 2020b). Present in coastal and marine environments worldwide, plastics are incorporated into all environmental compartments (air, water, sediment, sand, soil, biota) and affect roughly 700 known species to date—a number that continues to grow with the addition of new research (Provencher et al. 2017). MPs have been found in a variety of edible seafood species globally (e.g., Van Cauwenberghe & Janssen 2014; Li et al. 2015; Rochman et al. 2015; Davidson and Dudas 2016; Lusher et al. 2017; Phuong et al. 2018), suggesting that marine predators and even human consumers of these species are exposed to MPs; however there are also myriad other non-seafood human consumables known to

contain MPs including drinking water (e.g. Erkes-Medrano et al. 2018; Tong et al. 2020), table salt (e.g. Kim et al. 2018; Cho et al. 2019; Lee et al. 2019), milk (Kutralam-Muniasamy et al. 2020), honey (Liebezeit and Liebezeit 2013), beer (Liebezeit and Liebezeit 2014) and packaged meats (Kedzierski et al. 2020). With trophic transfer of MPs previously demonstrated in laboratory settings (Farrell and Nelson 2013; Nelms et al. 2018), there is little doubt that MPs are now inextricably incorporated into marine food webs and the human diet (Carbery et al. 2018; Barboza et al. 2018).

The Pacific Razor clam (*Siliqua patula*) is found on intertidal beaches along the Pacific coast of North America from Alaska to southern California (Link 2000). On the Olympic Peninsula, Washington (WA), United States (U.S.) which supports the largest population of harvest-sized individuals within the species' range, razor clams rely on the rich coastal waters to settle and grow (Huppert and Trainer 2014). This historically-important species is a key traditional food and cultural resource, and serves as a vital form of income for members of the Quinault Indian Nation and other Olympic Coast peoples (Crosman et al. 2019).

While there is a distinctive lack of data on the prevalence and effects of MPs in commercially-important North American fishery species (Baechler et al. 2020a), recent research indicates that Pacific Northwest bivalves from less developed coastal areas are not immune to MP contamination (Davidson and Dudas 2016; Covernton et al. 2019; Baechler et al. 2020b; Martinelli et al. 2020). Determining the prevalence of MPs in organisms from varied geographies provides context for environmental concentrations, bioavailability to marine species and potential risk to human consumers. Investigating

MP prevalence in marine and seafood species from relatively undeveloped coastal areas contributes to our understanding of the range of environmental and human exposure to MPs through seafood consumption in areas of relatively low human impact. MPs become incorporated into bivalves through ingestion or adherence to soft tissues (e.g., de Witte et al. 2014; Mathalon and Hill 2014; Kolandhasamy et al. 2018; Teng et al. 2019; Abidli et al. 2019), with potentially negative physiological impacts including increased respiration, altered reproduction and feeding, inflammation, neurotoxicity, and decreased energy reserves (e.g., von Moos et al. 2012; Avio et al. 2015; Sussarellu et al. 2016; Ribeiro et al. 2017; Xu et al. 2017; Bour et al. 2018).

We initiated this study on the sparsely populated Olympic Coast, WA, U.S. to determine: 1) microplastic burdens in Pacific razor clams from the Olympic Coast; 2) annual human microplastic exposure ranges from Olympic Coast razor clam consumption based on clam preparation styles, consumption frequency and consumer demographics; and 3) level of knowledge and concerns Olympic coast razor clam harvesters have regarding microplastic exposure from seafood.

We expected MPs would be present in Olympic Coast razor clams, as has been found in other filter-feeding bivalves (clams, oysters) in the region (e.g., Davidson and Dudas 2016; Covernton et al. 2019; Baechler et al. 2020b; Martinelli et al. 2020) and in a number of clam species globally (e.g., Li et al. 2015; Su et al. 2018; Abidli et al. 2019; Sparks 2020), but that MP burdens would be highest at northernmost sites due to outflow from Puget Sound and the Salish Sea– waterbodies adjacent to the populous Seattle metropolitan area abutting the Olympic Peninsula. This hypothesis was founded upon the



assumptions that densely populated areas can lead to high concentrations of MPs in the coastal marine environment, due to land-based inputs (Barnes et al. 2009; Hantoro et al. 2019), and that waterborne MP concentrations partially dictate bivalve MP burdens (Qu et al. 2018). We predicted Puget Sound and the Salish Sea would transport substantial waterborne MPs to the Olympic Coast, based on existing hydrographic models of water movement from the highly populated Salish Sea region to the sparsely populated northern Olympic Coast (Pirhalla et al. 2009) and MP transport in coastal littoral drift cells- pockets of water (and associated particulates or contaminants) of varying energy levels that move alongshore (Black et al. 2018; Horn et al. 2019).

We hypothesized annual Olympic Coast razor clam harvester-consumer MP exposure would vary depending on clam preparation style, ethnicity, and frequency of consumption. This hypothesis was based on the knowledge that some ethnicities consume more seafood per capita than the general population, resulting in greater exposure to seafood-associated contaminants (Sechena et al. 1999; Mahaffey et al. 2009; EPA 2014; Cisneros-Montemayor et al. 2016). Lastly, we hypothesized that razor clam harvesters would possess varying knowledge and concerns about MPs in seafood.

### **3.2 Methods:**

#### *Study area context*

"When the tide is out, the table is set" is a common saying on the Olympic Peninsula, the rugged coastal region which forms the western border of WA, U.S. (Charles et al. 2004). In this ecologically-productive area, as in others across North

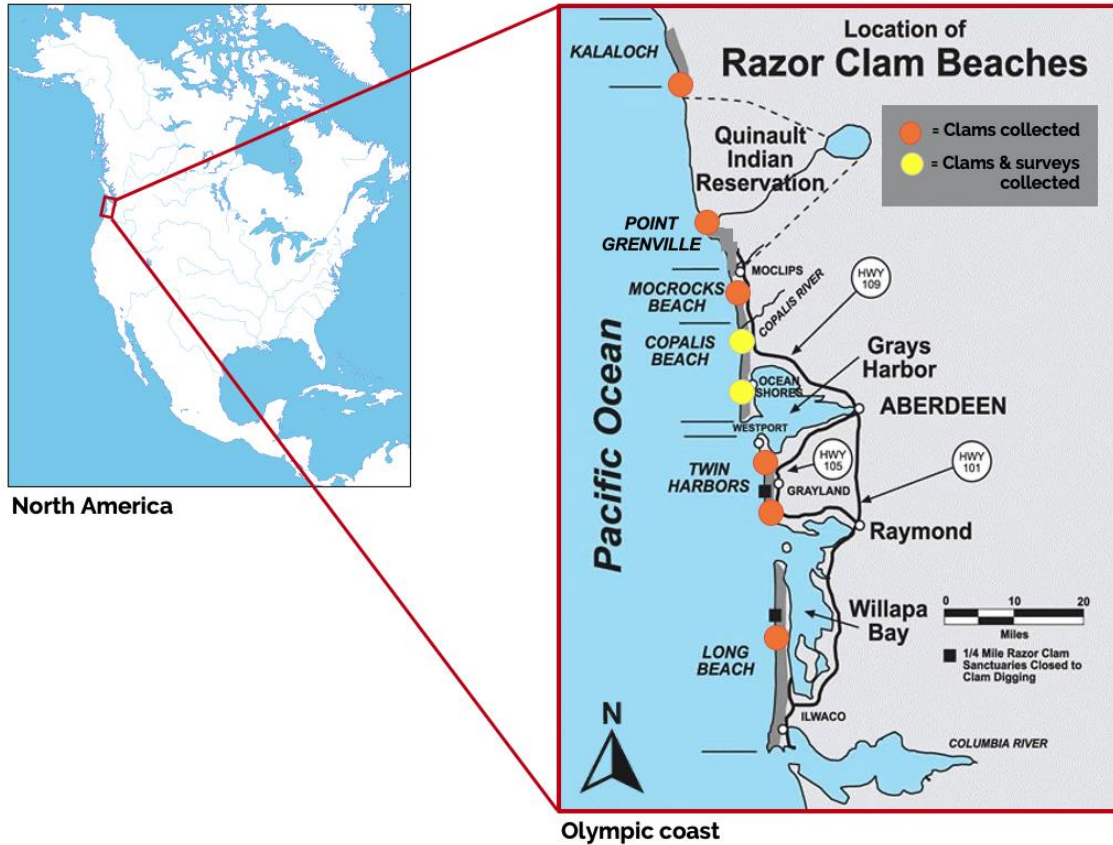
America, harvest of the nearshore seafood bounty by Native Americans has occurred since time immemorial and is significantly integrated into local indigenous culture, economy, gastronomy and food security (Olson 1936; Charles et al. 2004; Anderson and Parker 2009; Lepofsky et al. 2015).

The Olympic Coast is a region of low human presence relative to other coastal areas in North America. It is sparsely populated by about 195,000 people at a density of 12 people/km<sup>2</sup> (30 people /mile<sup>2</sup>) in coastal Jefferson, Clallam, Grays Harbor, and Pacific counties (U.S. Census Bureau 2012), and a very low proportion of developed lands compared to other land use types based on 2010 data (Appendix C: Chapter 3; NOAA 2020). For comparison, in the U.S., average coastal population density (excluding Alaska) was 446 people per square mile in 2010 (NOAA Office for Coastal Management 2020). While the west coast of the U.S. has the state with the highest coastal-dwelling population (California—26.5 million people), the east and gulf coasts have states with the second through fifth highest coastal populations (New York—16 million people; Florida—15.3 million people; New Jersey—7.1 million people; Texas—6.6 million people), providing evidence that coastal living is prized nationwide (Crossett et al. 2013; NOAA Office for Coastal Management 2020). Unfortunately, land development, particularly near densely populated coastlines, can impact coastal ecology through environmental degradation such as loss of habitat for native species, erosion, and pollution in various forms (nutrient, chemical, noise, light, plastic).

The allure of the razor clam attracts thousands of visitors to the Olympic Coast's sandy shores annually, as they try their luck at clamming during one or more recreational

fishery openers. The recreational fishery injects what are usually sleepy coastal towns with visitors, filling hotels and restaurants to capacity, and bringing millions of dollars annually into coastal community economies. Roughly 250,000 clam digger trips are logged in this region each year (Huppert and Trainer 2014).

Razor clams are co-managed in Washington by both the Department of Fish and Wildlife (WDFW) and by tribal governments (Wyer 2013). Tribal (ceremonial, subsistence and commercial) and state (recreational and commercial) fisheries have unique rules for who can participate and where harvest can occur. The Quinault Indian Nation is the sole manager and harvester of razor clams on beaches within their reservation boundaries (Figure 1). The Quinault Nation's treaty rights extend to beaches north and south of their reservation, within their legally defined "usual and accustomed fishing grounds" (U&A), where they co-manage razor clams with WDFW and other tribes. Fifty percent of total allowable harvest is reserved per treaty between the United States and the Quinault people on U&A areas off-reservation (shared with the Hoh Tribe on Kalaloch Beach to the north), with the remainder available for recreational harvest in the state-managed fishery (Anderson 1999). Commercial and subsistence razor clam harvest off-reservation is regulated by Quinault for tribal members at Copalis, Mocrocks, and Kalaloch beaches. Recreational harvest is permitted by WDFW on set harvest dates, and is open to anyone with a Washington shellfish harvest permit (Crosman et al. 2019).



**FIGURE 1.** Modified publicly-available map of recreational and commercial razor clam beaches on the Olympic Peninsula, Washington, with razor clam collection sites (orange circles) and sites where both razor clams were collected and clam harvester surveys were conducted (yellow circles) in April 2018. Sample beaches included (from North to South): Kalaloch beach, Point Grenville beach (within the Quinault Indian Reservation), Mocrocks Beach, Copalis Beach, Twin Harbors and Long Beach. Original map source: <http://wdfw.wa.gov/fishing/shellfish/razorclams/>.

*Microplastic prevalence in study species*

*Sample sites and collection*

Olympic Peninsula clams were collected from eight coastal beaches (Figure 1; Kalaloch, Point Grenville, Mocrocks, Copalis, South Copalis, North Twin Harbor, South Twin Harbor, and Long Beach) in April 2018 to quantify types and burdens of MPs in their tissues. Fifteen clams were collected at each of the eight sites, except at Mocrocks beach where only fourteen were collected due to challenging harvest conditions. Razor

clams sampled averaged  $69.90 \pm 2.63$ g wet body weight per individual (range= 4.66–167.02g); therefore, on average, roughly 1.05kg of razor clam tissue was collected per site (except at Mocrocks beach due to only fourteen clams being collected). Clams were not selected by size; rather, the first fifteen clams obtained per site were retained for analysis. Clams were transported in clean, covered 2L glass mason jars on ice to the Applied Coastal Ecology lab at Portland State University in Portland, Oregon (OR), where the exteriors of the organisms were rinsed with filtered deionized (DI; Shelco MicroVantage WGPS0.2-20S7S213 filtered to  $0.22\mu\text{m}$ ) water and biological measurements (shell length in mm, body tissue weight in g) were collected. Individual organisms were processed as either whole (n= 7 individuals for each site), gut-tissue separated (n= 3 individuals for each site, divided into 3 gut-only and 3 tissue-only samples), or cleaned samples (n= 5 per site). “Whole” organisms were minimally processed except for shells being rinsed, to mimic MP burdens if the clam was consumed by an animal predator. “Gut-tissue” samples (stomach, intestine, and crystalline style) were separated from “non-gut tissue” samples (all remaining tissue; Table 1). “Cleaned” samples were prepared as if to be eaten by a human consumer; in order to remove gritty sand and other debris inside of or stuck to the clam, the tip of the siphon was removed, and both the incurrent and excurrent siphons were split using cleaned stainless steel scissors and rinsed thoroughly under a running filtered DI water tap. The clam foot was also split and thoroughly rinsed under a running filtered DI water tap. Digestive organs and gills were removed using cleaned stainless steel scissors (Table 1). Each sample was rinsed into individual cleaned and labeled mason jars and frozen at  $-20^{\circ}\text{C}$  (Table 1). Of

the 138 clam samples analyzed in this study, 52 were whole, with others separated into gut and tissue samples (“gut-tissue”; n= 24, and “non-gut tissue”, n= 23 respectively), or fully cleaned (“cleaned”; n= 39).

**TABLE 1.** Descriptions of the four clam sample types included in this study.

Sample type	Sample type description	# of Samples	Avg # MP/sample	Range # MP/sample	MPs previously reported for this species and sample type?
Whole	Shell rinsed with filtered DI water; was minimally prepared as if consumed by an animal predator. Includes whole organism (including gut-tissue and nongut tissue)	52	6.75 ± 0.60	0–20	Yes; Baechler et al. 2020b
Gut-tissue	Shell rinsed with filtered DI water; includes stomach, intestine, and crystalline style	24	7.88 ± 0.71	3–16	Yes; Baechler et al. 2020b
Nongut-tissue	Shell rinsed with filtered DI water; includes all remaining tissue after gut-tissue was separated	23	4.96 ± 0.56	0–12	Yes; Baechler et al. 2020b
Cleaned	Shell rinsed with filtered DI water; was prepared as if to be eaten by a human consumer. The tip of the siphon was removed. The incurrent and excurrent siphons and foot were split using cleaned stainless steel scissors and rinsed thoroughly under a running filtered DI water tap. Digestive organs and gills were removed using cleaned stainless steel scissors	39	3.44 ± 0.25	0–6	No

*Notes:* DI= Deionized (filtered to 22 µm); MP= suspected microplastics; Avg= average; #= number. Error reported for Avg # MP/sample is Standard Error.

### Sample Processing

Samples were thawed and digested individually using the protocol described in Baechler et al. (2020b). In brief, samples were individually chemically digested using a 10% potassium hydroxide (KOH) solution, then underwent a second 10% KOH digestion combined with hypersaline density separation (330g/L Fisher Chemical Certified ACS Crystalline NaCl) and were visually analyzed for MPs under a Leica M165C stereomicroscope (10–120x magnification). Each suspected MP encountered was photographed with a Leica IC80 HD camera connected to a computer running Leica

Application Suite X imaging software. Particle category (bead, fiber, fiber bundle, film, fragment, foam, unknown), color, and maximum length were recorded. All clam sample types (whole, gut-tissue, non-gut tissue, cleaned) underwent identical procedures during collection, transport, digestion and microscope analysis. In a randomly-selected subset of approximately 10% of suspected MPs (53 total) extracted from whole and cleaned clams, material types were determined using a Thermo Nicolet is20 Fourier-transform infrared spectrometer (FTIR) and iN5 microscope fitted with an Attenuated Total Reflectance accessory and germanium tip running at 128–512 scans in the Brander laboratory at Oregon State University. Spectral outputs were compared to FTIR spectral libraries (Omic polymer database, Primpke et al. 2018; Primpke et al. 2020), with a 70% minimum match threshold to determine a quality spectrum and material type. Material type was determined by the match with the highest percentage.

#### Quality Assurance/Quality Control

MPs and other anthropogenic fibers (AFs) are airborne, waterborne, and present on surfaces, and are therefore inadvertently introduced to samples during laboratory processing or analysis. These AFs – MPs or otherwise – labeled ‘contamination’, are important to quantify, as they provide context for final particle counts. To minimize contamination, clams were immediately rinsed with filtered DI water and placed in cleaned, rinsed and covered 2L mason jars after field collection. One hundred percent cotton clothing and lab coats, as well as nitrile gloves were worn at all times in the laboratory during sample processing, chemical digestions, and microscope analyses.

Nitrile gloves were changed after every sample, after handling tools or touching surfaces. Glassware and tools were all rinsed 3 times with filtered DI water (0.22 $\mu$ m pore size) between uses, and glassware was covered with cleaned watch glasses at all times. To quantify contamination, two control types (procedural and microscope blanks) were used to provide a snapshot of AFs inadvertently introduced into clam samples during laboratory processing and analysis (Brander et al. 2020). Procedural controls were samples of 50ml filtered DI water run through the same chemical digestion and sieving steps as other sample types; microscope blanks were glass petri dishes filled with filtered DI water placed on the microscope base and left open to the air during visual inspection of samples for MPs (Baechler et al. 2020b; Appendix D: Chapter 3). One procedural control was processed during each of the four weeks of laboratory chemical digestions (n= 4), and one microscope blank was processed per sample immediately following visual microscope analysis of that sample (n= 145).

### Calculations and Statistical analyses

MPs in this study refers to post-digestion visually-identified fibers and few particles suspected to be plastics, a subset (10%) of which were validated using FTIR; while the validated subset was randomly selected, we cannot be certain of the material types or origins for all MPs in this study. To identify any differences in MP burden between sample sites or between sample types (whole, gut-only, tissue-only, and cleaned) analysis of variance (ANOVA) and post-hoc Tukey tests were conducted. Variables including the number of MPs per sample and per gram of tissue (MP g<sup>-1</sup> tissue) were



square root transformed to normalize data prior to conducting statistical analyses. Linear regression models were used to examine relationships between biological parameters (shell length, body tissue wet weight) and MP burdens (per whole individual and  $g^{-1}$  tissue) and were expressed as number of MPs per sample or  $g^{-1}$  tissue (wet weight; whole organisms only). The statistical cutoff ( $\alpha$ ) for all tests was 0.05 with standard error (SE) reported.

#### *Razor clam harvester questionnaire*

##### Questionnaire sites and protocol

Written questionnaires were administered to recreational razor clam harvesters 18 years of age or older after concluding clamming activities at Mocrocks and Copalis beaches on April 21–22, 2018 (Figure 1). Frequencies of razor clam consumption, preparation type, number of individual razor clams consumed per meal, and demographics of respondents were determined using these questionnaires (Appendix E: Chapter 3). Estimates of recreational razor clam harvester MP exposure were calculated based on self-reported razor clam consumption frequencies. While not central to research questions, data on consumption of other non-razor clam marine seafood species were also collected to provide further context to study results (Appendix F: Chapter 3).

The questionnaire surveying effort occurred during state-managed recreational fishery openings on two days in April and not during tribal commercial or subsistence fishery opening dates. Thus, local tribes (Quinault, Quileute, Hoh, and others) were poorly represented in the survey. Questionnaires were administered within two hours of

low tide each day, with surveys beginning at 10:00am (low tide at 11:38am) and 11:00am (low tide at 12:30pm) on April 21 and 22, respectively. Three teams of two surveyors dispersed along the beach to cover as large an area as possible. Surveyors monitored the shoreline for every sixth clam harvester party that visually indicated they had completed clamming activities (i.e., turning their backs to the shore and walking towards the vehicle line carrying clam bag, shovel and any other equipment or personal effects). Surveyors intercepted a clam harvesting party, provided a brief description of the research project and asked if a representative of the party would be willing to complete a questionnaire. Upon agreement, they were provided a clipboard with the questionnaire and a pencil attached. Respondents wrote in answers themselves unless they requested for surveyors to write as they dictated. Responses were at-will (not required) except the first question indicating the respondent was 18 years of age or older (Appendix E: Chapter 3); therefore, not all respondents answered every question.

#### *MP exposure calculations and statistical analyses*

MP exposure ranges of Olympic Coast razor clam harvester-consumers from razor clam consumption were calculated by multiplying minimum and maximum positive consumptive responses (i.e., smallest nonzero consumption frequency) per season (winter, spring, summer, fall) by average suspected MP burden for whole or cleaned clams across all eight sample sites. These exposure ranges include a combination of MPs and fibers of unknown origins, based on FTIR results for randomly-selected fibers in whole and cleaned clam samples. Seasonal totals were combined to generate MP

exposure estimates. For our purposes, each season was represented evenly in three-month (13.05 week) blocks.

Questionnaire data were plotted using Microsoft Excel and the R-studio statistical program (v1.2.1335). ANOVA, post-hoc Tukey tests, and Welch's T-tests were run using the `.aov`, `tukey.test` and `t.test` functions in R-studio (R Core Team 2020) to identify differences in MP exposure among demographic groups based on gender, age, income, ethnicity or education level. Standard error (SE;  $\alpha=0.05$ ) was reported with averages. Razor clam consumption by ethnicity was reported but not analyzed for statistical differences due to small sample size for some ethnicities; instead, a Welch's t-test was performed to elucidate differences in razor clam consumption between White/Caucasian and all other ethnic groups. For the single open-ended questionnaire item "Please expand on what concerns you have about microplastics in your food", responses were categorized into seven themes based on the short, written responses provided (Table 3).

### 3.3 Results:

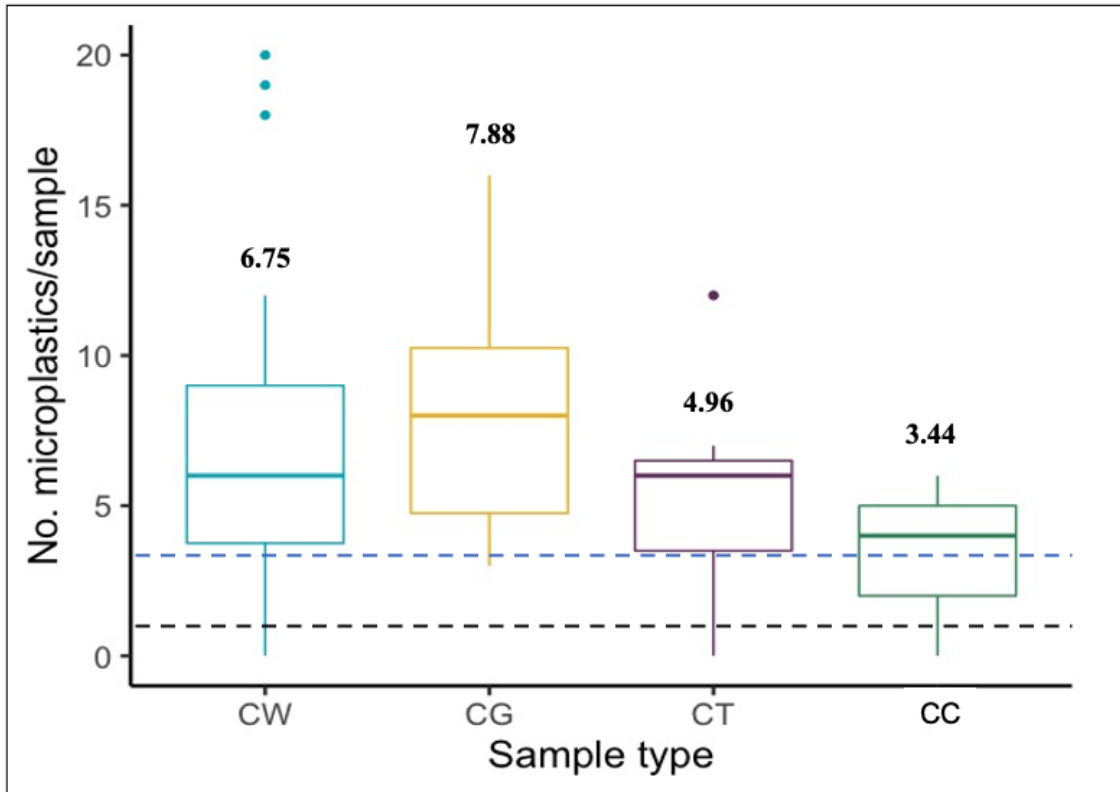
#### *Biological measurements*

Razor clams averaged  $115.71 \pm 1.29$ mm shell length (range= 63.91–156.76mm) and  $69.90 \pm 2.63$ grams (g) wet body weight (range= 4.66–167.02g), as the first 15 clams harvested per site were retained regardless of size. Average clam size was similar for all sites except for the northernmost Kalaloch beach, where clams were significantly smaller than other sites for both shell length (avg=  $102.85 \pm 2.25$ mm; range= 81.38–114.88mm;  $F_{7,43} = 4.00$ ,  $p = 0.002$ ) and wet body weight (avg=  $32.45 \pm 2.12$ g; range= 15.37–45.10g;  $F_{7,44} = 7.60$ ,  $p < 0.0001$ ).

#### *Microplastic characteristics and identification*

Suspected MPs were found in razor clams at all eight sample sites. In total, 799 suspected MPs were found in the 138 clam samples collected during this study. Over 99% of suspected MPs encountered were microfibers (n= 793) with an average length of  $1.33 \pm 0.04$ mm (range= 0.11–7.84mm; Appendix G: Chapter 3). Other categories represented were: unknown (n= 3), fiber bundles (n= 2), and fragments (n= 1). Colorless, blue, gray, and black were the most commonly observed colors at 55.19% (n= 441), 19.27% (n= 154), 12.27% (n= 98) and 4.63% (n= 37), respectively (Appendix H: Chapter 3). FTIR analysis of 53 randomly-selected suspected MPs (10.83% of suspected MPs encountered in whole clams and 10.45% of suspected MPs encountered in cleaned clams) yielded reliable matches for 52 fibers and verified that a total of 80.77% of the 52 fibers with matches were either synthetic (48.08%, n= 25), including polyethylene terephthalate (n= 21), polyester (n= 3), and nylon (n=1), or semi-synthetic (32.69%; n=17), including

cellulose acetate (n= 11) and cellophane (n= 6; Appendix I: Chapter 3, Appendix J: Chapter 3). Cellulose, a naturally-derived material, was also identified (n=10; 19.23%). One additional suspected MP was analyzed but its spectral match did not meet the 70% minimum threshold; material type for that fiber was unconfirmed and reported as “low spectral match”. The average match used for identification was  $86.6\% \pm 1.0\%$ .

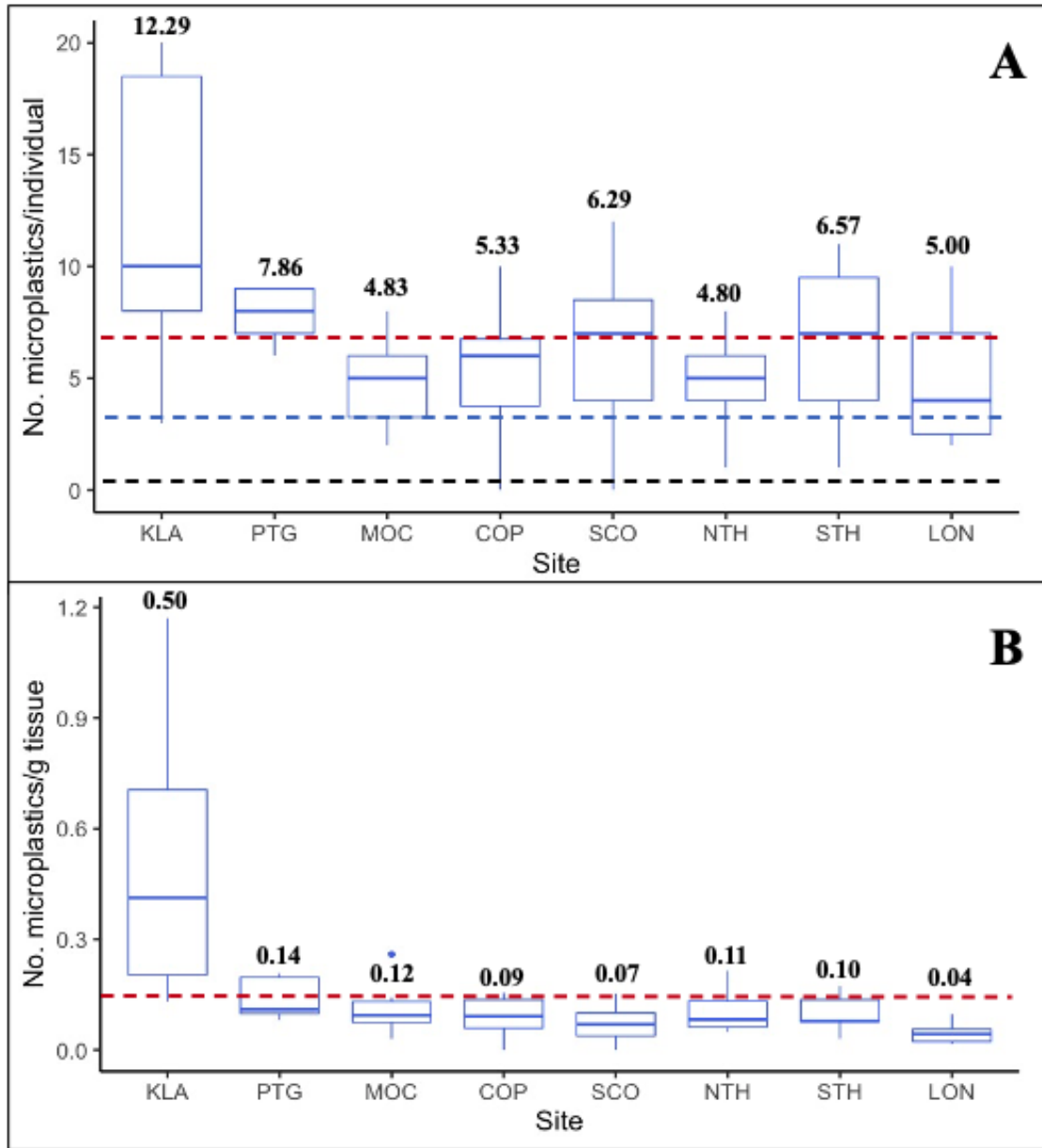


**FIGURE 2.** Number of microplastics (MPs) by sample type. CW= whole clam (n= 52); CG= gut-tissue only (n= 24); CT= tissue-only (n= 23); CC= cleaned clam (n= 39). No.= number. Black hashed line indicates average microscope blank contamination (Avg= 0.23 MPs/sample; n= 145). Blue hashed line indicates average procedural control contamination (Avg= 3.25 MPs/sample; n= 4). Bold numbers above boxes indicate average number of MPs per sample type.

### *Microplastics prevalence*

Whole clams and gut-tissue samples contained more MPs per sample than cleaned clams ( $p = 0.0004$  and  $p < 0.0001$ , respectively), and gut-tissue samples contained

significantly more MP than non-gut tissue ( $p = 0.017$ ; Figure 2). Across all sites, average whole clam suspected MP burden was  $6.75 \pm 0.60$  (range= 0–20,  $n = 52$ ; Figure 3A). For gut-tissue, non-gut tissue, and cleaned samples, average MP burdens across all sites were  $7.88 \pm 0.71$  ( $n = 24$ ; range= 3–16),  $4.96 \pm 0.56$  ( $n = 23$ ; range= 0–12), and  $3.44 \pm 0.25$  ( $n = 39$ ; range= 0–6), respectively. When comparing the number of MPs in all four sample types (Table 1) across all sites, significant differences were identified ( $F_{3,134} = 9.858$ ,  $p < 0.0001$ ). For whole clams, average MP  $g^{-1}$  burden was  $0.15 \pm 0.03$  (range= 0–1.17,  $n = 52$ ; Figure 3B). MP  $g^{-1}$  tissue differed by site, with higher MP  $g^{-1}$  tissue found at the northernmost Kalaloch site than all other sites ( $F_{7,44} = 7.171$ ,  $p < 0.0001$ ). Site-specific differences in MP  $g^{-1}$  tissue were identified between Kalaloch and: Copalis ( $p = 0.0002$ ), Long Beach ( $p < 0.0001$ ), Mocrocks ( $p = 0.002$ ), North Twin Harbor ( $p = 0.003$ ), South Copalis ( $p < 0.0001$ ), South Twin Harbor ( $p = 0.0006$ ), and Point Grenville ( $p = 0.01$ ; Figure 3A-B). In contrast to differences in MP  $g^{-1}$  tissue, the number of MPs per clam differed minimally across sites ( $F_{7,44} = 1.892$ ,  $p = 0.09$ ). Number of MPs did not differ among sites for gut-tissue ( $F_{7,16} = 1.095$ ,  $p = 0.41$ ) or non-gut-tissue samples ( $F_{7,15} = 1.076$ ,  $p = 0.42$ ), but did for cleaned clams ( $F_{7,31} = 3.020$ ,  $p = 0.02$ ) for two site pairings: Copalis and South Copalis ( $p = 0.01$ ) and South Copalis and North Twin Harbor ( $p = 0.04$ ). Neither clam shell length (in mm;  $F_{1,49} = 0.941$ ,  $p = 0.34$ ) nor body weight (g wet weight;  $F_{1,50} = 0.010$ ,  $p = 0.92$ ) correlated with the number of MPs per whole organism.



**FIGURE 3 (A–B):** Number of microplastics (MPs)- (A) Per individual whole razor clam, with red line indicating average number of MPs for all eight sites (Avg= 6.75 MPs/individual), black hashed line indicating average microscope blank contamination (Avg= 0.23 MPs/sample; n= 145), and blue hashed line indicating average procedural control contamination (Avg= 3.25 MPs/sample; n= 4); and (B) Per gram of tissue (wet weight), with red line indicating average number of MPs per gram of tissue (wet weight) for all eight sites (Avg= 0.15 MP g<sup>-1</sup> tissue). Bold numbers above boxes indicate average number of MPs per sample type. No.= number. Sites are arranged north to south by latitude. KLA= Kalaloch beach (n= 7), PTG= Point Grenville (within the Quinault Indian Reservation boundary; n= 7), MOC= Mocrocks Beach (n= 6), COP= Copalis Beach (n= 6), SCO= South Copalis Beach (n= 7), NTH= North Twin Harbor (n= 5), STH= South Twin Harbor (n= 7), LON= Long Beach (n= 7).

### *Quantifying microplastic contamination*

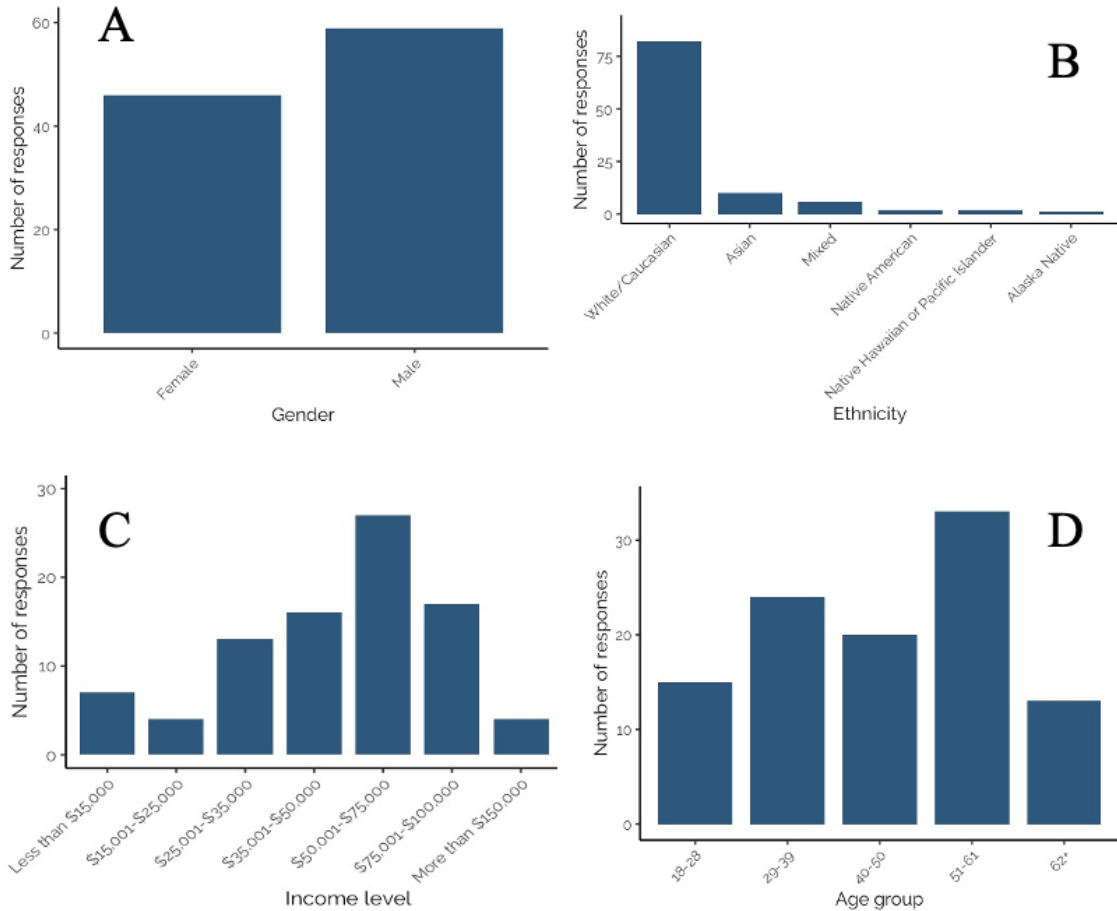
In total, 46 suspected MPs were identified as contamination from microscope blanks (n= 145) and procedural controls (n= 4). On average, suspected MP contamination in microscope blanks was  $0.23 \pm 0.05$  (range= 0–2), and in procedural controls was  $3.25 \pm 0.85$  (range= 1–5; Figure 2). Average MP length detected as contamination was  $1.60 \pm 0.15$  mm (range: 0.12–3.6mm). One hundred percent (100%) of suspected MPs in microscope blanks and procedural controls were microfibers and were colorless (77.8%; n= 35), blue (15.6%; n= 7), black (4.4%; n= 2), or gray (2.2%; n= 1). Neither microscope blank nor procedural control MPs was subtracted from clam MP burdens because procedural controls were low in number and were intended to identify a range of contamination during laboratory processing, with microscope blanks showing a range of contamination during microscope analyses. Reported MP burdens in the four clam sample types may be lower than reported given ambient contamination found in microscope blank and procedural control samples. Some cleaned razor clams contained fewer MPs than the maximum contamination found in our controls; in these cases, our clam MP burdens may also be higher than would be expected in the environment.

### *Questionnaire respondent demographics*

The two-day survey effort at Mocrecks and Copalis beaches yielded 107 questionnaires from recreational razor clam harvesters representing an array of ethnicities, incomes, educational backgrounds, and ages. Questionnaire response rate was 94.7%, as 6 of the 113 individuals asked to participate declined. Respondents were 56.2%



male, 43.8% female (Figure 4A), and lived in 14 Washington counties including Benton (n= 3), Chelan (n= 1), Clallam (n= 2), Clark (n= 4), Franklin (n= 1), Grays Harbor (n= 6), King (n= 24), Kitsap (n= 5), Lewis (n= 3), Mason (n= 6), Pierce (n= 17), Skagit (n= 3), Snohomish (n= 12), and Thurston (n= 15). None of the 107 respondents dug razor clams to sell commercially in April 2017–April 2018. Roughly 96% (n= 103) of questionnaire respondents reported their ethnicities. The majority of respondents (79.6%) were White/Caucasian, and the remaining (20.4%) were Asian, mixed race, Native American, Native Hawaiian or Pacific Islander, or Alaska Native (Figure 4B). Due to small sample size for most ethnic groups surveyed (White/Caucasian individuals: n= 82; Asian: n= 10; Mixed ethnicity: n= 6; Native American: n= 2; Native Hawaiian or Pacific islanders: n= 2; and Alaska Native: n= 1); therefore, differences in razor clam consumption were analyzed between White/Caucasian (79.6% of respondents) and all other ethnic groups (Non-White/Caucasian; 20.4% of respondents). Most respondents reported an annual income range of \$50,001–\$75,000 U.S. dollars (Figure 4C). Respondent ages were lumped into groups; the youngest respondents were 18–28 years and the oldest were 62+ years, but most (31.4%) were 51–61 years of age (Figure 4D).



**FIGURE 4 (A–D):** From top left to bottom right– Number of Olympic Coast recreational razor clam harvester questionnaire responses by: (A) gender; (B) ethnicity; (C) income level; and (D) age group. Bold numbers above bars represent number of responses.

### *Razor clam harvest and consumption*

While only 83.8% of questionnaire respondents reportedly consumed razor clams in the past year (n= 88), all (100%; n= 107) participated in recreational razor clamming activities during the survey period (April 21–22, 2018). Razor clams consumed by respondents were obtained in a variety of ways; the majority (76.0%; n= 76) harvested the clams they ate, while some individuals obtained their clams in multiple ways (11.0%; n= 11), got clams from friends or family (4.0%; n= 4), purchased them (2.0%; n= 2), or didn't know or obtained clams in other ways (3.0%; n= 3). The average number of razor

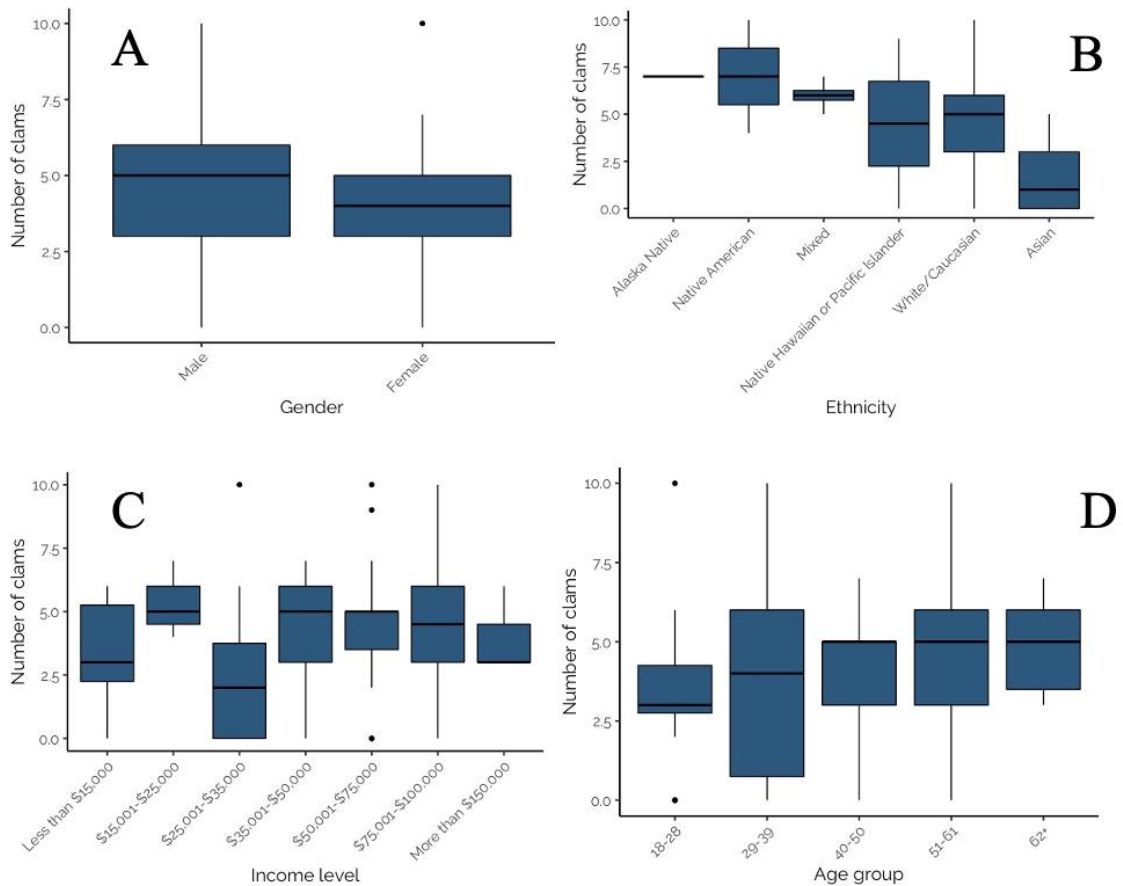
clams consumed per meal was  $4.27 \pm 0.27$  clams (range= 0–10); however, most respondents consumed either three or five clams per sitting (23.3% or 18.9%, respectively).

The majority of respondents (88.4%; n= 91) consumed razor clams without guts, while some did not know if they consumed the gut or not (9.7%; n= 10). One individual reported sometimes consuming them with, and sometimes without guts (1.0%; n= 1), and one individual reported consuming them with guts (1.0%; n= 1). More respondents reported consuming clams in the spring (81.1%; n= 77) than winter (72.8%; n= 67), fall (70.4%; n= 62), or summer (57.1%; n= 48) seasons.

To elucidate patterns in clam consumption, we considered demographic factors including gender, ethnicity, income and age group. The number of clams consumed per sitting varied by gender (Figure 5A), with males consuming significantly more than females (males:  $4.81 \pm 0.37$ , females:  $3.78 \pm 0.34$ ;  $T = -2.051$ ,  $p = 0.04$ ). Clam consumption also varied by ethnicity, but these differences were not statistically analyzed due to low response numbers (as low as n= 1) for some categories (Figure 5B). On average, Alaska Native and Native American respondents consumed the highest number of clams per sitting at 7.0 (n= 1) and  $7.0 \pm 3.0$  (n= 2), respectively. The next highest consumers of razor clams per sitting were Mixed ethnicity individuals ( $6.0 \pm 0.40$  clams; n= 4), followed by Native Hawaiian or Pacific islanders ( $4.50 \pm 4.50$  clams; n= 2), White/Caucasians ( $4.46 \pm 0.26$  clams; n= 69), and Asians ( $1.56 \pm 0.60$ ; n= 9). No difference was found when comparing number of clams consumed per meal for White/Caucasian and Non-White/Caucasian (all ethnicities except White/Caucasian;  $T = -$

0.841,  $p=0.41$ ). The number of clams consumed per sitting did not vary by respondent income level (Figure 5C;  $F_{7,73}=1.047$ ,  $p=0.41$ ) or age group (Figure 5D;  $F_{4,83}=0.924$ ,  $p=0.45$ ).

In addition to razor clams, respondents reported consuming a variety of other common marine shellfish including bivalves (oysters, mussels, bay clams, hard shell clams, geoducks, scallops), crustaceans (Dungeness crab, other crab, lobster), gastropods (snails), and echinoderms (urchins; Appendix F: Chapter 3).



**FIGURE 5** (A–D): From top left to bottom right– Number of razor clams consumed per sitting by Olympic Coast recreational razor clam harvester: (A) gender; (B) ethnicity; (C) income level; and (D) age group. Significant differences were identified between males and females (panel A;  $p=0.04$ ), but not by income level ( $p=0.41$ ) or age ( $p=0.45$ ).

*Razor clam harvester-consumer microplastic exposure*

Using a combination of our razor clam MP burdens and questionnaire data, we estimated annual ranges of MP exposure through razor clam consumption by recreational harvester-consumers on the Olympic Coast (Table 2, Figure 6, Figure 7A–B).

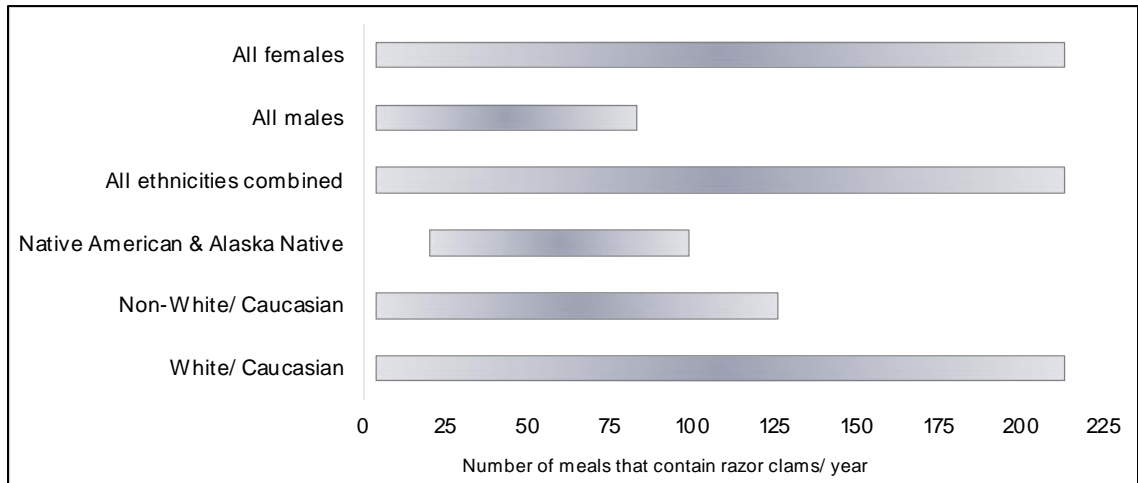
**TABLE 2.** The estimated annual microplastic exposure ranges by Olympic Coast, WA razor clam harvester-consumers. MP exposures were rounded to the nearest 10. The number of razor clams consumed per sitting, and minimum and maximum number of razor clam meals consumed per year were self-reported. Average number of microplastics for whole and cleaned razor clams were determined through analyses of clams collected from eight Olympic Coast sites. Minimum razor clam meals consumed include nonzero responses only.

Consumer group	Respondents (#)	Avg # clams consumed /sitting	Min clam meals consumed /yr	Max clam meals consumed /yr	CLEANED CLAMS <sup>1</sup>		WHOLE CLAMS <sup>2</sup>	
					Min MP exposure/yr <sup>3</sup>	Max MP exposure/yr <sup>4</sup>	Min MP exposure/yr <sup>3</sup>	Max MP exposure/yr <sup>4</sup>
All females	46	3.78	4	209	50	2,720	100	5,330
All males	59	4.81	4	79	70	1,310	130	2,570
All ethnicities combined	105	4.27	4	209	60	3,070	120	6,020
Native American & Alaska Native	3	7.00	20	79	480	1,900	950	3,730
Non-White/Caucasian	21	3.78	4	122	50	1,590	100	3,110
White/Caucasian	82	4.46	4	209	60	3,210	120	6,290

*Notes:* “Clam”= Pacific razor clam, Avg= Average, MP= Suspected microplastics, yr= Year, min= minimum, max= maximum; <sup>1</sup>= Calculated using Avg suspected MP burden of 3.44 MP/clam, <sup>2</sup>= Calculated using Avg suspected MP burden of 6.75 MP/clam, <sup>3</sup>= Avg # clams consumed per sitting x Avg # MP/clam x Annual min razor clam meals consumed, <sup>4</sup>= Avg # clams consumed per sitting x Avg # MP/clam x Annual max razor clam meals consumed.

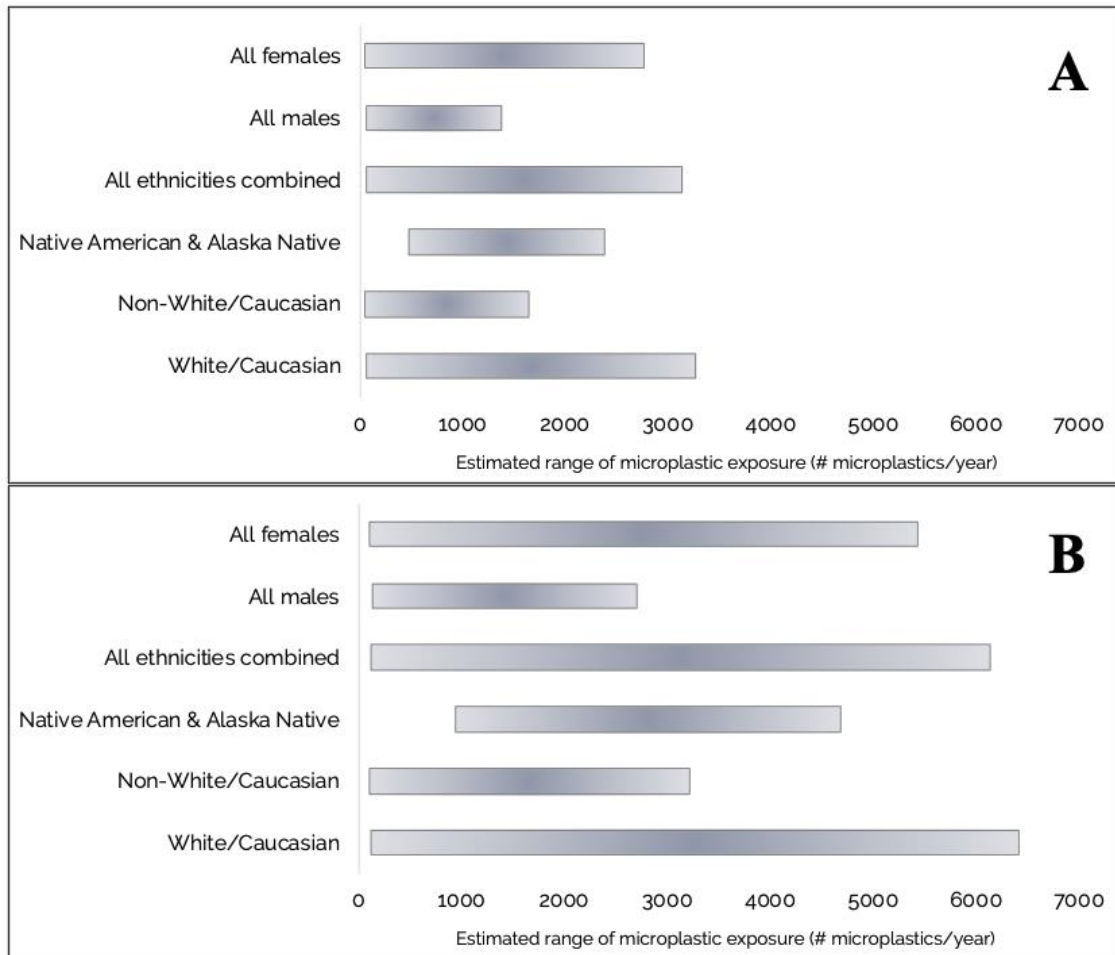
Of the questionnaire respondents, 16.2% (n= 17) harvested but did not consume razor clams in a given year, but those that did (83.8%; n= 88) consumed between 4 and 209 razor clam meals per year (Table 2, Figure 6). Based on minimum and maximum number of razor clam meals per year and average suspected MP burdens in cleaned and whole clams, we estimated the range of MP exposure from razor clams by Olympic Coast harvester-consumers to be 60–3,070 MP/yr for those that thoroughly clean their clams before consuming them, or 120–6,020 MP/yr for those that consume clams whole without removing guts, gills or other organs (Table 2). Despite males consuming more clams per

meal than females, female razor clam harvesters exhibited a broader range of exposure to MP due to higher maximum reported clam consumption than males in winter, summer and fall seasons (Table 2).



**FIGURE 6.** Ranges for the number of razor clam meals consumed per year by Olympic Coast harvester-consumers: Females (n= 46), Males (n= 59), All ethnicities combined (n= 87), Native American & Alaska Native (n= 3), Non-White/Caucasian (includes all ethnicities except White/Caucasian; n= 18), and White/Caucasian (n= 69).

Microplastic exposure from razor clam consumption was also broken down by ethnicity into: All (all ethnicities), Native American & Alaska Native, Non-White/Caucasian (all other ethnicities excluding White/Caucasian), and White/Caucasian groups (Table 2, Figure 7A–B). The three Native American and Alaska Native respondents in our sample consumed more clams per sitting than other respondents; therefore the minimum exposure to MP through razor clam consumption for this group is higher than for the other ethnicities analyzed (Table 2, Figure 7A–B).



**FIGURE 7 (A–B):** Ranges of estimated annual Olympic Coast razor clam harvester-consumer microplastic (MP) exposure for: Females (n= 46), males (n= 59), all ethnicities combined (n= 87), Native American & Alaska Native (n= 3), Non-White/Caucasian (n= 18), or White/Caucasian (n= 69) respondents from consumption of: (A) Cleaned razor clams with the foot and siphons cleaned and rinsed and the siphon tips, digestive organs and gills removed; and (B) Whole razor clams.

### *Questionnaire respondent knowledge & concerns about microplastics in food*

When asked “how familiar are you with the concept of microplastics in food?”, 64.8% (n = 68) of respondents said they were not familiar, 28.6% (n= 30) said they were moderately familiar, and 6.7% (n= 7) said they were very familiar. When asked “do you have concerns about microplastics in your food”, 41.9% (n= 44) indicated yes, 39.0% (n= 41) indicated no, and 19.0% (n= 20) did not know. Twenty-five respondents (39.0% of

those that indicated ‘Yes’ or ‘I don’t know’ for the question “do you have concerns about microplastics in your food”) answered the open-ended question “Please expand on what concerns you have about microplastics in your food”. Concerns recorded were categorized into seven themes: Impacts on humans (n= 6), General concern (n= 5), Health, chemicals and toxins (n= 4), Impacts on humans and the environment (n= 4), Uncertainty or unknown (n= 3), Impacts on the environment (n= 2), and Management (n= 1; Table 3).

**TABLE 3.** Themes elucidated from Olympic Coast razor clam harvester questionnaire survey responses (n= 25) for the question “Please expand on what concerns you have about microplastics in your food”.

Impacts on Humans	General Concern	Health, Chemicals and Toxins	Impacts on Humans and the Environment	Uncertainty, Unknown	Impacts on Environment	Management
Cancer-causing? Do we need to be worried? Long-term effects?	Just general concern	Health Hazards	Biomagnification	Haven't studied enough to know	Whole environment concerns	What they're going to do about it
Don't want to eat them, and accumulation	Garbage in ocean	General health	Biomagnification	I don't know enough	How it affects the food chain	
Don't want in food	General presence	Toxins, health concerns	Effects on the whole planet	Not sure what it is		
Drinking water	They are out there	Any chemicals	Health of environment and humans			
Not cool with that stuff in my food, to be honest	In water					
Not something I want to ingest						

### 3.4 Discussion:

This study confirms MP presence in Olympic Coast Pacific razor clams, a species important to the PNW’s coastal ecology, culture, food security, and economy. Razor clams represent not only a traditional but also a critical present-day food source for members of the Quinault Indian Nation and other Olympic Coast peoples. As the

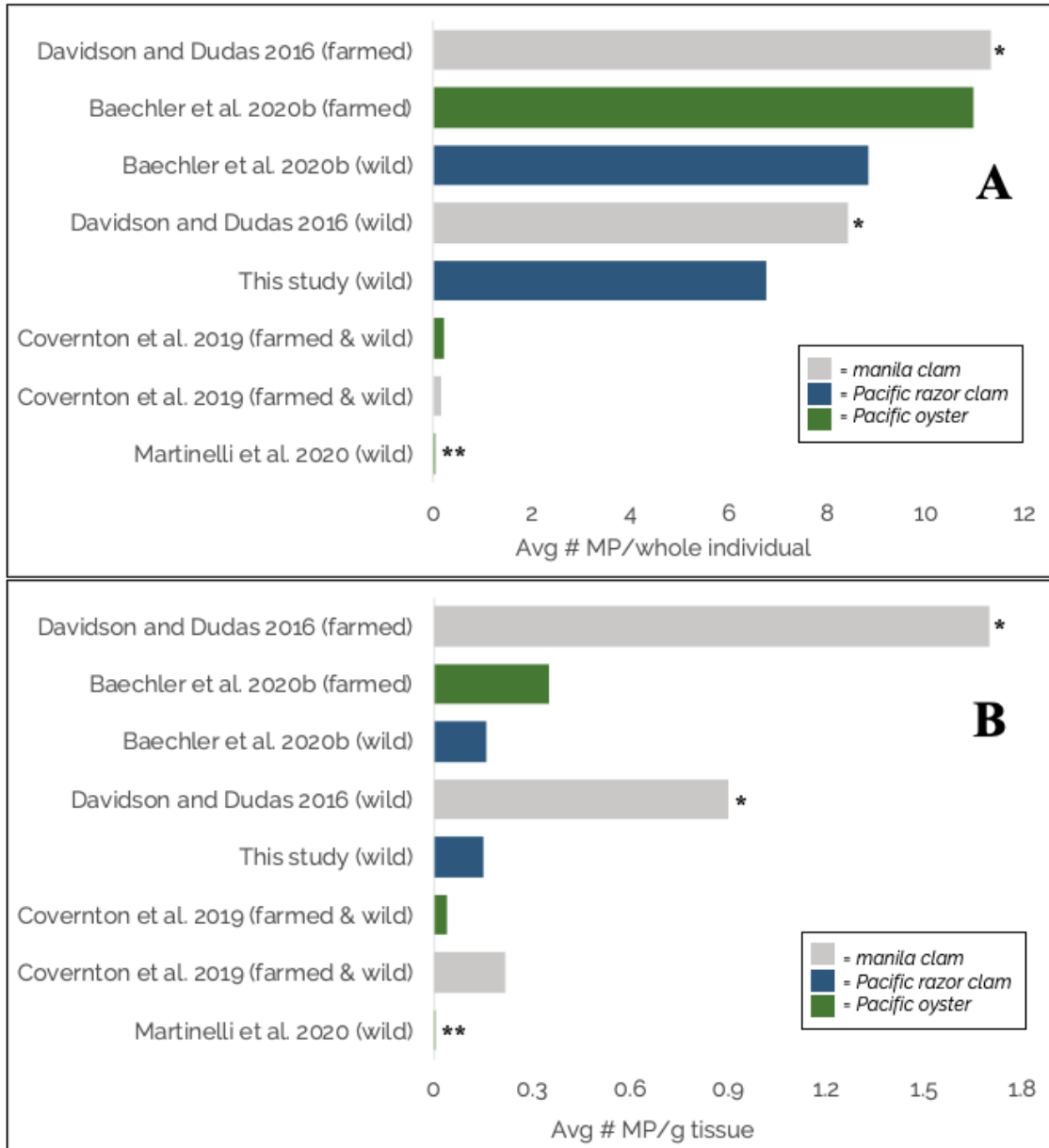


Olympic Peninsula is sparsely populated relative to other (non-Alaskan) coastal areas in the U.S., this study provides a snapshot of MP prevalence in an area of relatively low human influence. A number of existing studies have found that elevated environmental MP concentrations can be attributed to proximity to dense human population (Barnes et al. 2009; Hantoro et al. 2019); therefore, this study may represent the lower end of human MP exposure from Pacific razor clam consumption in the continental U.S.

The highest MP  $\text{g}^{-1}$  tissue site average was from our northernmost study site, Kalaloch beach (Figure 4B). There are no major rivers near the Kalaloch sample site to deliver large loads of MPs here relative to the other seven sites; however, Kalaloch was the northernmost of our eight sites and is closest in proximity to the highly populated Salish Sea region and at the mouth of Puget Sound. We predicted substantial waterborne MPs would be transported from Puget Sound and the Salish Sea to the Olympic Coast, based on existing hydrographic models (Pirhalla et al. 2009). Proximity of these large water bodies to the Kalaloch site could contribute to its higher-than-average MP concentrations in razor clams. 2010 land cover data for the four coastal Olympic Peninsula counties (Clallam, Jefferson, Grays Harbor, Pacific) show forests are the most dominant land type, followed by scrublands (NOAA 2020; Appendix C: Chapter 3). Development and agriculture represent a small proportion of land use in these counties (Appendix C: Chapter 3) and are likely not the primary dictator of MP burdens in razor clams collected from these sites; therefore, we expect factors other than land use cover are responsible for Kalaloch clams containing significantly more MPs than clams at other sites. Kalaloch clams were the smallest, on average (both shell length and body weight),

of all eight study sites. While we did not find a correlation between MPs and body size (shell length or body weight) across study sites, clam size may have contributed to high MP g<sup>-1</sup> tissue at Kalaloch, as mass-specific filtration can be faster in smaller bivalves relative to their larger counterparts (e.g., Powell et al. 1992; Ehrich and Harris 2015).

Average MP burdens per whole razor clam and g<sup>-1</sup> tissue in this study fell roughly mid-range compared to MP burdens reported in other bivalves (manila clams, Pacific razor clams, Pacific oysters) throughout the region: the Salish Sea, WA (Martinelli et al. 2020), British Columbia, Canada (Davidson and Dudas 2016; Covernton et al. 2019), and the Oregon coast (Baechler et al. 2020b; Figure 8). It should be noted that these studies employed varying methodologies for MP extraction, identification, and reporting, suggesting the need for caution in interpreting direct comparisons. Methodological variations included differences in digestion chemicals (KOH, H<sub>2</sub>O<sub>2</sub>), microplastic isolation methods (density separation, sieving, vacuum filtration), MP g<sup>-1</sup> tissue calculations (use of wet weight vs dry weight), and validation techniques (none, Raman, or FTIR spectroscopy; Figure 8).



**FIGURE 8 (A–B):** Comparisons of average microplastic burdens in Pacific Northwest bivalves (Washington and Oregon, U.S. and British Columbia, Canada), in– (A) Average number of microplastics per individual; and (B) Average number of microplastics per gram of tissue (wet weight; except dry weight for Covernton et al. 2019). Gray bars indicate manila clams, blue bars indicate Pacific razor clams, green bars indicate Pacific oysters. Avg= Average, MP= microplastic, g= gram. \*=All studies except Davidson and Dudas included suspected microplastic material validation using Fourier Transform Infrared (FTIR) or Raman spectroscopy. \*\*= For general context only; calculated from Table 2 of Martinelli et al. (2020) as average of site-specific visually-identified number of particles per oyster (panel A) or per gram of tissue (panel B) for 10 sites x average site-specific percentage of suspected microplastics confirmed to be plastic through Raman spectroscopy for 10 sites.

Using FTIR we determined that the randomly-selected suspected MPs analyzed (n=53) were plastic, semi-synthetic, and natural, with 80.77% confirmed to be either synthetic or semi-synthetic (Kroon et al. 2018; Appendix I: Chapter 3). Polyethylene terephthalate (PET), a member of the polyester family and a widely used thermoplastic polymer resin in clothing and drink containers, was the most commonly identified synthetic material (n=21; Appendix I: Chapter 3, Appendix J: Chapter 3). PET fibers are ubiquitous in coastal areas globally and are often detected in bivalves (Mishra et al. 2019; Hope et al. 2020). The most commonly identified semi-synthetic fiber was cellulose acetate (n=11; Appendix I: Chapter 3, Appendix J: Chapter 3), a material found in cigarette filters and clothing, that is commonly detected on beaches and in waterways (Miller et al. 2017; Mishra et al. 2019). Cellulose was the lone natural material present in our subsample (n=10; Appendix I: Chapter 3).

Due to our methodology, the lower size limit of detectable MPs was 63 $\mu$ m, though smaller MPs and nanoplastics (NPs; <1 $\mu$ m) are known to migrate into the tissues and organs of marine organisms (Lusher et al. 2017). While methodologically, any suspected MPs greater than 63 $\mu$ m in size were included in our samples, the smallest MP we identified was 110 $\mu$ m. Therefore, MPs and NPs smaller than 110 $\mu$ m may have been present but undetected in our samples. Additionally, it is unclear if the MPs identified in our whole, cleaned or nongut-tissue samples were incorporated into clams through ingestion or adherence to soft tissues, as both avenues of incorporation have been reported in bivalves (e.g., Xu et al. 2017; Kolandhasamy et al. 2018).

Combining ecological data on razor clam MP burdens with structured questionnaires allowed us to calculate MP exposure ranges for the Olympic Coast razor clam harvester-consumers surveyed. MP exposure calculations included natural cellulose particles representing 19.23% of suspected MPs based on FTIR material confirmation results; therefore exposure ranges to MPs may be overestimates. Regardless, our findings suggest the harvester-consumers in this study are likely exposed to low levels of confirmed MPs (relative to other known sources of ingested MPs). Razor clams are unique in that, unlike many other clam and oyster species which are generally consumed whole, they are typically cleaned with parts removed prior to consumption. The Quinault Indian Nation Department of Fisheries suggests the Quinault and other coastal tribal peoples clean razor clams prior to consumption, and the Quinault commercial razor clam processing plant in Taholah similarly cleans clams prior to sale to eliminate guts and gills (Ervin “Joe” Schumacker, Quinault Indian Nation Department of Fisheries, pers. comm. May 1, 2020). While most questionnaire respondents fully cleaned razor clams before consuming them, 11.7% either consumed the gut or weren’t sure if they did. Based on our findings, removal of the gut prior to consumption for this particular species reduces MP burden by nearly 50% and may be a prudent measure to reduce consumer MP exposure from this particular seafood source. While cleaned clams may substantially reduce MP exposure for human consumers, this approach is not an option for marine razor clam predators.

There are currently no established safety thresholds for human MP intake; however, there are established thresholds for domoic acid in Olympic Coast razor clams.

Fishery openings and closures have recently been impacted by the presence of domoic acid, a neurotoxin that can cause direct and severe harm to human consumers, including affected memory when consumed in low doses over long periods of time (Chadsey et al. 2012; Grattan et al. 2016; Ferriss et al. 2017; Lefebvre et al. 2017). The Washington Department of Health regularly tests clams for the presence of domoic acid, and closes fisheries when the level meets or exceeds 20 parts per million (ppm; Chadsey et al. 2012; WDFW 2020). In 2016, the state of Washington issued a public health advisory on razor clam consumption, recommending no more than 15 clams be consumed per month, due to the propensity of razor clams to contain low levels of domoic acid below the Department of Health action level of 20ppm (Washington Department of Health 2016).

Microplastics ingested by humans from seafood can be significant in some geographic areas (e.g., Li et al. 2015); however, MP exposure of our study group from razor clam consumption is low relative to other expected exposure routes such as inhalation (estimated maximum of 30,000,000 MP/year; Zhang et al. 2020a), deposition of microfibers during meals (estimated 13,731–68,415 MPs/year; Catarino et al. 2018), consumption of table salt (estimated maximum of 73,000 MPs/year; Zhang et al. 2020a) or drinking bottled water (estimated 90,000 MPs/year; Cox et al. 2019). Our estimates of Olympic Coast razor clam harvester-consumer MP exposure are lower than existing estimates of dietary MPs through bivalve consumption in Europe (~11,000 MP/year; Van Cauwenberghe & Janssen 2014) and China (~110,000 MP/year; Li et al. 2015). This result is expected considering the Olympic Coast is sparsely populated (roughly 12 people/km<sup>2</sup> or 30 people/mile<sup>2</sup>; U.S. Census Bureau 2012) and Southeast Asia and

Europe support some of the most densely-populated coastal areas in the world (>10,000 people/km<sup>2</sup>; Nicholls and Small 2002). Moreover, our estimates are for a single species that is frequently cleaned prior to consumption.

Although the vast majority of MPs orally ingested by humans are thought to be excreted (EFSA CONTAM Panel 2016), some proportion of particles may remain in the body. While effects of MPs on human health are not well known, MPs may cause harm due to blockage, release of associated additives, or liberation of sorbed chemicals (Wright and Kelly 2017). Inhaled MPs may become trapped in the lung, purged from the body (i.e., from coughing or sneezing) or, depending on size, enter the digestive tract (Wright and Kelly 2017; Cox et al. 2019). Differential effects from consuming versus inhaling microplastics may be present, though this area is understudied and warrants additional research.

Ferriss et al. (2017) estimated the frequency of razor clam consumption by WA coast recreational razor clam harvesters in 2015-16 at 6.0 clams per day, on days that clams were eaten. Males consumed, on average, more clams/day ( $6.5 \pm 0.8$ ) than females ( $5.4 \pm 0.6$ ), with higher overall consumption in the spring than in other seasons (Ferriss et al. 2017). While not directly comparable to our results because we report consumption in number of clams per sitting ( $4.27 \pm 0.27$ ) instead of per day, the pattern of males consuming more than females, and seasonal clam consumption being highest in spring relative to other seasons are similar. An Olympic Coast razor clam domoic acid study by Grattan et al. (2016), placed those that consume 15 or more razor clams per month in a “high” consumer group, and those that consume fewer than 15 per month into a “low”

consumer group based on the 2016 WA-recommended consumption limit of 15 clams per month (Washington Department of Health 2016). According to our findings, “high” consumers (2.67%, n=7 responses in our study) would then be exposed to a minimum of roughly 600 MP/yr if thoroughly cleaning 15 clams per month before consuming them, or a minimum of 1,200 MP/yr if consuming 15 clams per month without cleaning them. Exposure values would increase proportionally with the number of clams consumed per month. Due to the low sample size of Alaska Native and Native American respondents (n=3) in this study, it is not possible to broadly generalize about clam consumption frequencies in either group; however, responses from these few individuals were striking considering the much higher minimum exposure level of MPs through razor clam consumption than other ethnicities, due to a higher minimum number of meals containing razor clams eaten annually and more clams consumed per sitting than other groups. Due to this finding and the knowledge that seafood consumption tendencies vary by ethnicity (e.g. Sechena et al. 1999; Mahaffey et al. 2009; EPA 2014; Cisneros-Montemayor et al. 2016), additional research regarding microplastic exposure from seafood consumption for these and other high-seafood consumer demographics is both warranted and necessary.

In conclusion, MP presence in the PNW coastal environment and in food webs, raises concern about potential for ecological harm to Pacific razor clams, their predators, and innumerable other marine species. There is an immense need to reduce future transmission of microplastics, and in particular microfibers, to the marine environment, as these are commonly found in biotic and environmental samples (Barnes et al. 2009; Barboza et al. 2018). A focus on land-based solutions to microplastic pollution is



imperative, as more than 80% of primary and secondary microplastics originate on land (Miranda et al. 2019). Apparel washing is a significant vector of synthetic microfibers to the environment; since the 1950's, over 5.9 million metric tons of synthetic microplastics have been generated through laundering (Gavigan et al. 2020). Our findings of primarily microfibers in the guts and tissues of Olympic Coast razor clams highlight the need to develop effective upstream pollution control solutions that capture this type of pollution at the source. One solution to significantly reduce transmission of microfibers into the environment would be the integration of microplastic capture technologies into washing machines. These technologies prevent synthetic microfibers from flowing through household graywater to wastewater treatment plants, where they settle and are frequently extracted along with biosolids and applied to agricultural soils as fertilizer (Mahon et al. 2017; Gavigan et al. 2020). Fiber catchment-integrated washing machines have already been deployed in Japan and, per legislation, will be required in France beginning in 2025 (European Parliament 2020). Broader adoption of similar technologies in the U.S. and around the world could drastically reduce global laundry microfiber emissions in the future.

In conclusion, we identified MPs in razor clams from all of our coastal study sites, highlighting the ubiquity of MPs along the Olympic Coast. The Olympic Coast recreational razor clam harvester-consumers represented in this study were from 14 WA counties and encompassed an array of ethnicities, incomes, education levels, and concerns about the concept of microplastics in food. This work improves understanding of MP prevalence in an important and desirable edible shellfish species. It highlights that

MPs are present in seafood even in relatively low-impact areas; however, when put into context, this specific seafood item represents a minimal vector of MPs for the recreational razor clam harvester-consumer group studied. Our work serves as an important reference in the growing portfolio of MP research in the PNW to inform future MP attenuation. It can contribute to estimations of overall human MP exposure and could aid in the development of human health standards for this pollution type.

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## **Chapter 4: Aquarium Visitor Engagement with an Ocean Plastic Exhibit: Effects on Self-Reported Single-Use Plastic Reductions and Plastic-Related Environmental Stewardship Actions**

*Special note:* This manuscript was first submitted to the journal Environmental Education Research in Oct. 2020 and is currently in review.

### **4.1 Introduction:**

Plastic is one of the most heavily relied-upon materials used by humans today. Derived from petroleum, plastics are lightweight, durable, inexpensive, and versatile (Andrady 2011; Cole et al. 2011; Eriksen et al. 2014; Auta et al. 2017). From food wrappers and drink containers, to bottles for cleaners and personal products, electronics, textiles, furniture, vehicles, and building components, plastics are integrated into every facet of our existence. Large-scale plastic production began in the 1950's, has increased exponentially since, and will likely continue along this trajectory in the coming decades (Napper and Thompson 2020). Plastics have many important uses and benefits; however, overproduction and mismanagement have led to a plastic pollution problem which has escalated into a pervasive environmental crisis (Barnes et al. 2009; Jambeck et al. 2015; Vince and Stoett 2018).

It is estimated that in 2016, up to 23 million metric tons of plastic waste entered the aquatic environment worldwide (Borelle et al. 2020). A growing body of literature supports the thesis that environmental plastics pose more than just aesthetic harm; they impact human communities (Phelan et al. 2020) and are regularly encountered by wildlife, causing direct harm through entanglement or ingestion (Kühn et al. 2015). As

plastics permeate the environment, the need to reduce our plastic dependency— particularly on problematic single-use items— grows more pressing. Unfortunately, there are no simple solutions. Instead, multifaceted approaches including reductions in plastic production, improved waste management, transitioning to a more circular economy, and increasing the scale of environmental recovery are all needed (Veiga et al. 2016; Lau et al. 2020; Borelle et al. 2020). While regulatory actions, infrastructure improvements and interventions such as capture devices and cleanups are critical tools with substantial potential to help tackle this global issue (e.g., Hohn et al. 2020; Lau et al. 2020), reducing individual-level consumption of problematic single-use plastic items is another essential component of the solution as individual actions influence the global waste stream. Individual behavioral shifts replacing single-use plastic items with reusable alternatives can collectively help diminish plastic waste and leakage into the environment (Schnurr et al. 2018). Consumer behaviors leading to plastic pollution that cause environmental degradation are often unintentional; thus, there is immense possibility to promote habits and create positive, long-term behavioral change through education (Knussen and Yule 2008; Ohtomo and Ohnuma 2014; Pahl and Wyles 2017).

As is documented in the literature, sustainability-related behavioral shifts can be difficult to elicit, but effective education and outreach campaigns can empower people and ultimately turn the desired behaviors into actions (e.g., Chase et al. 2009; Leisher et al. 2012; Korkala et al. 2014; Sujata et al. 2019; Napper and Thompson 2020). While environmental stewardship and sustainability topics can and should be approached in the classroom, informal learning is a powerful way to disseminate information to broad

audiences. Aquaria and zoos provide excellent opportunities for visitor empowerment and education with respect to natural history, living organisms, and environmental issues. Educational exhibits and programs at these types of facilities stimulate conservation interest, awareness, enthusiasm and advocacy (Falk and Adelman 2003; Briseño-Garzón et al. 2007; Jensen et al. 2017). Visitors flock to aquaria and zoos to spend time with family and friends, satisfy their curiosity, experience a fun outlet for intellectual stimulation, and challenge their existing knowledge (Falk and Adelman 2003; Johnson and Kubarek 2019). Over 200 million people visit Association of Zoos and Aquariums facilities around the globe annually, with U.S. facilities hosting roughly 183 million people each year (Association of Zoos & Aquariums 2020). Families or family groups typically represent over half of all visitors to zoos and aquaria (Diamond 1986; Blud 1990; Briseño-Garzón et al. 2007); these visits help families build values, interests, and a collective family identity (Ellenbogen et al. 2004).

Exhibits and programming encountered in informal learning settings can leave lasting impressions and influence long-term behaviors, particularly when relevant to visitors' prior knowledge or experience, communication materials are clearly and convincingly written, the conservation goals are linked to everyday actions, and examples of how to achieve the actions are presented (Ballantyne et al. 2007). Positive effects from visits to zoos and aquaria have been shown to persist for years post-visit (Jensen et al. 2017). In particular, exhibits and displays that offer interactive elements can be highly impactful, as they stimulate learning and increase understanding of the topic at hand (Cohen 1987).

Studying human behavior is important to inform education and outreach strategies and effective approaches for environmental problem solving (Pahl and Wyles 2017). Analyzing the amount of time visitors spend at an exhibit, how they engage with different exhibit elements, and determining what they like and dislike about the exhibit, can help guide exhibit designs that effectively engage and inform diverse audiences. One quantitative method to determine how well an exhibit holds a visitor's attention is by recording the amount of time spent in the exhibit. Other measures to quantify engagement with exhibits (e.g., number of hands-on interactions, number of glances) can be used as complimentary proxies for visitor interest in the exhibit and specific exhibit elements.

We designed and installed an exhibit at the Oregon Coast Aquarium (OCA), located in Newport, Oregon to educate visitors about ocean plastic pollution. The exhibit featured written information in panel format and hands-on learning opportunities regarding ocean plastic prevalence, sources to the environment, types of plastic materials, ways marine organisms are affected by microplastics, and how individuals can reduce plastic transmission into the environment. Plain language was used wherever possible to avoid scientific jargon, which can hinder understanding of scientific concepts and generate distrust in the concepts presented (Bullock et al. 2019; Shulman et al. 2020).

To assess exhibit engagement, visitor knowledge about ocean plastic, learning outcomes, exhibit strengths and potential areas of improvement, and to evaluate existing and intended single-use plastic actions of OCA visitors, we asked the following research questions:

- 1. How does engagement differ between interactive and visual exhibit elements and by visitor gender, age, and exhibit crowding level?**

- 2. What baseline knowledge do visitors possess about ocean plastic, and did this exhibit facilitate new learning?**
- 3. Did the exhibit generate an intent to change behaviors including single-use plastic reduction or plastic stewardship actions and did this vary by demographic (gender, age, zip code)?**
- 4. What do visitors (and their children, if applicable) like most about the exhibit, and how can it be improved?**

For question 1, we hypothesized interactive elements would foster greater engagement from children (0–17 years) than adults (18–80+ years), and visual elements would foster greater engagement from adults than children. We predicted visitors would spend more time in the exhibit during the less crowded (November) than the more crowded (August) season, but it would not differ by gender. For questions 2 and 3, we hypothesized: 1) Visitors would have some baseline knowledge about the ocean plastic problem, considering OCA visitors have previously prioritized plastic pollution as a conservation problem (Johnson and Kubarek 2019), and aquarium visitors tend to have higher-than-expected competency about basic environmental concepts when compared to other groups (Reinhard et al. 2007); 2) Visitors would garner new information from the exhibit; and 3) Plastic use behavior changes would differ by age, residence in states with marine coastlines, and in Oregon residents vs non-Oregonians.

#### **4.2 Materials & Methods- Study site, exhibit design and evaluation:**

Oregon Coast Aquarium (OCA)



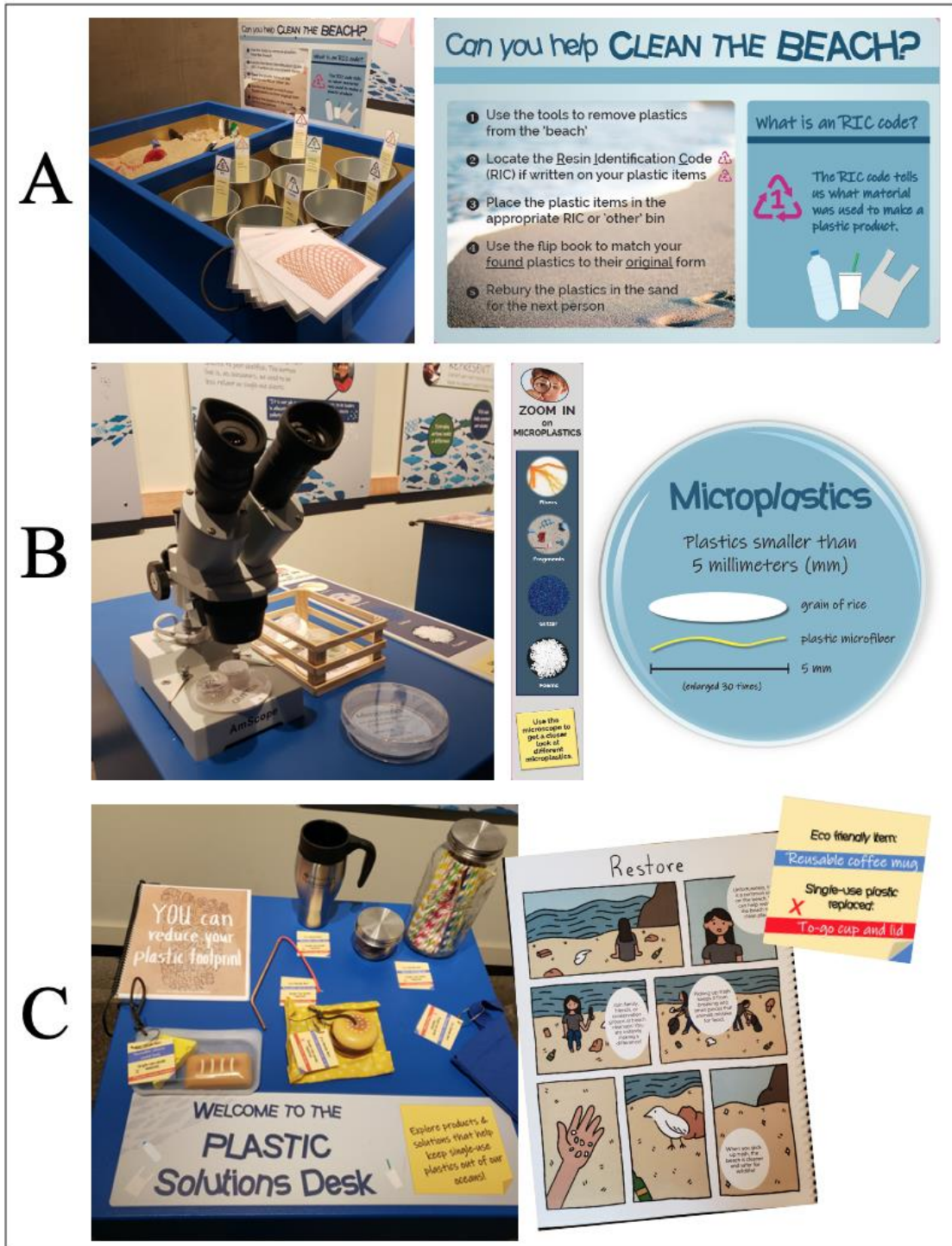
The OCA, a nonprofit organization located in Newport, Oregon, hosts over 420,000 visitors annually (Kerry Carlin-Morgan, personal communication August 2020). The Aquarium’s mission is to “create unique and engaging experiences that connect you to the Oregon Coast and inspire ocean conservation”. In a 2019 study by Johnson and Kubareck, 260 OCA visitors were given a list of important conservation issues to prioritize including plastic pollution, climate change, ocean acidification, sustainable fisheries and illegal wildlife. OCA visitors listed addressing plastic pollution as the top conservation priority. OCA’s existing educational programming highlights ocean plastic pollution and how individuals can help combat the issue. At the time of installation on March 15, 2019, the only other ocean plastic-themed exhibit at OCA was an art piece displaying marine debris collected during a 2-hour cleanup on an Oregon beach.

### Exhibit design

Our ocean plastic exhibit consisted of four informational wall panels, three interactive exhibit enhancements mounted on wooden box stands, and a hanging art installation (Figures 1 & 2, Table 1, Appendix K: Chapter 4, Appendix L: Chapter 4). Editable electronic versions of the signs, exhibits, and products created for this exhibit are available as supplemental materials and can be adapted for future use.



**FIGURE 1.** Photo of the ocean plastic exhibit displayed at the Oregon Coast Aquarium (OCA) in Newport, Oregon, U.S. from March 2019–April 2020.



**FIGURE 2 (A-C):** Layout and content of the three ocean plastic exhibit interactive podia: A) *Can You Help Clean the Beach?* B) *Zoom in on Microplastics*, and C) *Plastic Solutions Desk*.

**TABLE 1.** Descriptions of ocean plastic exhibit informational panels, interactive elements, and art installation.

INFORMATIONAL PANELS	INTERACTIVE ELEMENTS	ART INSTALLATION
<p><i>Panel 1: Our plastic problem</i></p> <p>Introduced the plastic problem, using global numbers of plastic prevalence use and leakage into the environment. Ex: "8 million tons of plastic enter the oceans each year. That's equal to the weight of 40,000 blue whales!"</p>	<p><i>Clean the beach</i></p> <p>Included a divided sandbox mounted to a wooden box stand with a variety of plastic items buried in the sand, and a laminated flipbook. On each page of the flipbook were pictures of a fragment or piece of an everyday plastic item found on the table, and on the back were zoomed-out versions of those plastic items with the Resin Identification Code (RIC) codes noted. Each plastic item had a visible RIC code that visitors matched up to the plastic item it originated from. Directions included a short description and definition of RIC codes and instructions to: 1) Use tools to remove plastics from the 'beach'; 2) Locate the Resin Identification Code (RIC) on your plastic items; 3) Place your plastic items in appropriate RIC bins; 4) Use the flip book to match your found plastics to their original form; and 5) Reset for the next person: re-bury plastics in the sand.</p>	<p><i>Hanging shell art</i></p> <p>The piece was fabricated from a variety of locally-collected bivalve shells, resin, plastic pieces, and marine fishing line as an artistic interpretation of the global plastic pollution problem by Hanna Gentile and Anna Young. It was hung directly above the marine plastics display out of reach from museum visitors due to its fragile nature.</p>
<p><i>Panel 2: Plastic in the Environment</i></p> <p>Described how large pieces of plastic break up into microplastics, defined microplastics, and introduced the concept that marine organisms often mistake microplastics for food items.</p>	<p><i>Zoom in on microplastics</i></p> <p>Featured an adjustable dissecting microscope and two handheld magnifying glasses mounted to a wooden box stand. Photo descriptions of different microplastic types (fragments, foams, glitter, fibers) were listed on the interpretive panel next to the microscope. Samples of each microplastic type were available in small acrylic jars for observation under the microscope or with magnifying glasses. The instructions explained to visitors: "Peer through the microscope to discover what different types of microplastics look like up close!".</p>	
<p><i>Panel 3: Microplastics in coastal critters</i></p> <p>Provided information on Oregon-specific microplastics research investigating plastic prevalence in two edible Oregon bivalve species.</p>	<p><i>Plastic solutions desk.</i></p> <p>Featured a variety of alternatives to single-use plastic household items mounted to a wooden box stand, including glass mason jars for food storage, paper and metal straws, canvas tote bags, beeswax food wrap (and wooden food items to practice using the wrap), silicone food storage bags, insulated metal beverage mugs, and a cartoon booklet depicting plastic-reducing behaviors. Each plastic alternative was labeled with a tag identifying the plastic-free item and the single-use plastic item it could replace.</p>	
<p><i>Panel 4: It's time for solutions</i></p> <p>Provided suggestions for ways to reduce individual plastic footprints and provided examples of how to achieve solutions to the marine plastics problem using six "R" words: Refuse, Reduce, Repurpose, Recycle, Restore, and Represent.</p>		

To answer our first research question, we conducted timed interaction surveys to determine which exhibit elements were most and least successful at engaging visitors. To answer the second and third research questions, we administered a structured questionnaire to randomly-selected adults (18+ years) to determine if visitors were taking away the main messages of the exhibit, quantify changes to respondent interest in helping tackle the ocean plastic pollution problem, and document intended reductions in single-use plastic or plastic stewardship actions. Exhibit evaluation occurred over eight days during August and November 2019. All evaluation methods and questionnaire items were approved by Portland State University (PSU)'s Human Research Protection Program, Office of Research Integrity Internal Review Board (IRB) protocol # 196534-18. Timed interaction surveys and structured questionnaires were administered 11:00am–3:59pm on weekdays and weekends to control for possible time-of-day or day-of-week visitor differences (Appendix L: Chapter 4, Appendix N: Chapter 4). August represented the high-traffic season and November the low-traffic season. Questionnaires were developed with input from previous OCA exhibit summative assessments and surveys (Kathryn Owen Consulting 2015; Nalven 2019).

#### *Timed interaction surveys*

Timed interaction surveys assessed engagement with interactive and visual exhibit elements, by quantifying total time spent in the immediate exhibit area and number of times each exhibit element was read or physically touched (Appendix P: Chapter 4). Visitors were randomly selected for observations as they entered the exhibit (Appendix K: Chapter 4, Appendix N: Chapter 4). Timed interactions began at the top of

the hour between 11:00am–3:59pm, with up to 10 individuals timed per hour. Interactions of every third individual entering the exhibit space were timed during the August (high-traffic) survey, and those from every other individual timed during the November (low-traffic) survey. Time spent was recorded as the number of seconds in the exhibit space; timing began when the individual entered the exhibit space (crossed the imaginary exhibit entrance line; Appendix K: Chapter 4) and ended when they turned away. In instances where an individual turned away from the exhibit, exited the exhibit area, and returned, the time spent after returning was not added to the original time spent. Visitors that did not at least glance at the exhibit while walking through the exhibit area were not included in the survey. Researchers estimated visitor age ranges (0–5, 6–10, 11–17, 18–29, 30s, 40s, 50s, 60s, 70s, 80+) and genders (Male, Female, Unknown; Appendix P: Chapter 4). Timed interaction data were collected unobtrusively by one or two surveyors obscured from view at the surveyor post (Appendix K: Chapter 4, Appendix P: Chapter 4).

#### *Structured questionnaires*

Structured questionnaires with mixed question types (Yes/No/I don't know, Likert scale, Single-word response, Open-ended short-answer response) measured immediate impacts of the exhibit experience by assessing if visitors learned from the information presented in this exhibit, what they (and their children, if applicable) liked most about the exhibit, how they thought the exhibit could be improved, and which of 10 single-use plastic reduction or plastic stewardship actions they participate (or intend to participate) in. Questionnaires were administered to adult visitors that spent at least 30 seconds

engaging with the exhibit (Appendix K, M, O: Chapter 4). Individuals appearing younger than 16 years old and those who did not engage with the exhibit for 30 seconds or more were not asked to complete a questionnaire. The 30 second cutoff was determined by questionnaire survey administrators to be the approximate minimum number amount of time needed for a visitor to sufficiently view all four exhibit panels and interactive elements. The surveyor stood at the surveyor post obscured from view (Appendix K: Chapter 4). As target individuals began to turn away from the exhibit, the surveyor approached them, briefly described the project and asked for their consent to complete the questionnaire. An initial set of questions about exhibit perceptions, visitor knowledge about ocean plastic, and learning from the exhibit were posed orally, with the questionnaire administrator recording answers (Appendix N: Chapter 4; survey pages 1-2). The second set of questions about existing single-use plastic behaviors, intent to change behaviors and demographics (gender, age, zip code) were written in by respondents (Appendix N: Chapter 4; survey pages 3-4). After reporting frequency of the 10 single-use plastic reduction or plastic stewardship actions they currently participate in on a scale of 0-4 (0= Never; 1= Rarely-I have done this a couple of times; 2= Sometimes-about once or twice a week; 3= Frequently-three or more days a week; 4= Almost every day), respondents were asked to revisit the actions and place a single star next to actions they “probably will start doing or increase” after seeing the exhibit, two stars next to the actions they “definitely will start doing or increase” after seeing the exhibit, or no stars if they did not anticipate changing their activity level for that action after seeing the exhibit.

#### *Data analysis and coding*

Data were plotted using Microsoft Excel and R studio statistical package (R Core Team 2020). ANOVA, post-hoc Tukey tests, and Welch's T-tests were run using the .aov, tukey.test and t.test functions in R studio to identify statistically significant differences among groups ( $\alpha = 0.05$ ). Standard errors (SE) were reported with averages. Timed interaction survey data were analyzed by demographic (age and gender) and temporal (season, day of week) factors. Structured questionnaire data were analyzed according to the variables of respondent age, gender and zip code. Mean, median and mode values were calculated for each of the 10 single-use plastic reduction or plastic stewardship actions (Appendix S: Chapter 4). Open-ended questionnaire responses (Questions 3, 5, 6, 7, 9A, 10, 13, and 14; Appendix N: Chapter 4) were coded thematically by the Applied Coastal Ecology (ACE) lab group at Portland State University. The ACE group determined response themes and binned them accordingly. Each single-word response (Questions 13 and 14; Appendix N: Chapter 4) was binned into a single theme, but each short-answer response (Questions 3, 5, 6, 7, 9A, 10; Appendix N: Chapter 4) could be placed into more than one category if multiple themes were elicited. ACE thematic categorizations were reviewed by the paper's lead author and reorganized if necessary. Final categorizations were determined by the lead author. Percent agreement scores between the ACE lab and lead author were generated for each question based on categorization before and after final lead author determination, to quantitatively determine the extent to which the lead author's categorizations aligned with categorizations of other ACE scientists (Appendix Q: Chapter 4).



### 4.3 Results:

#### *Timed interaction survey*

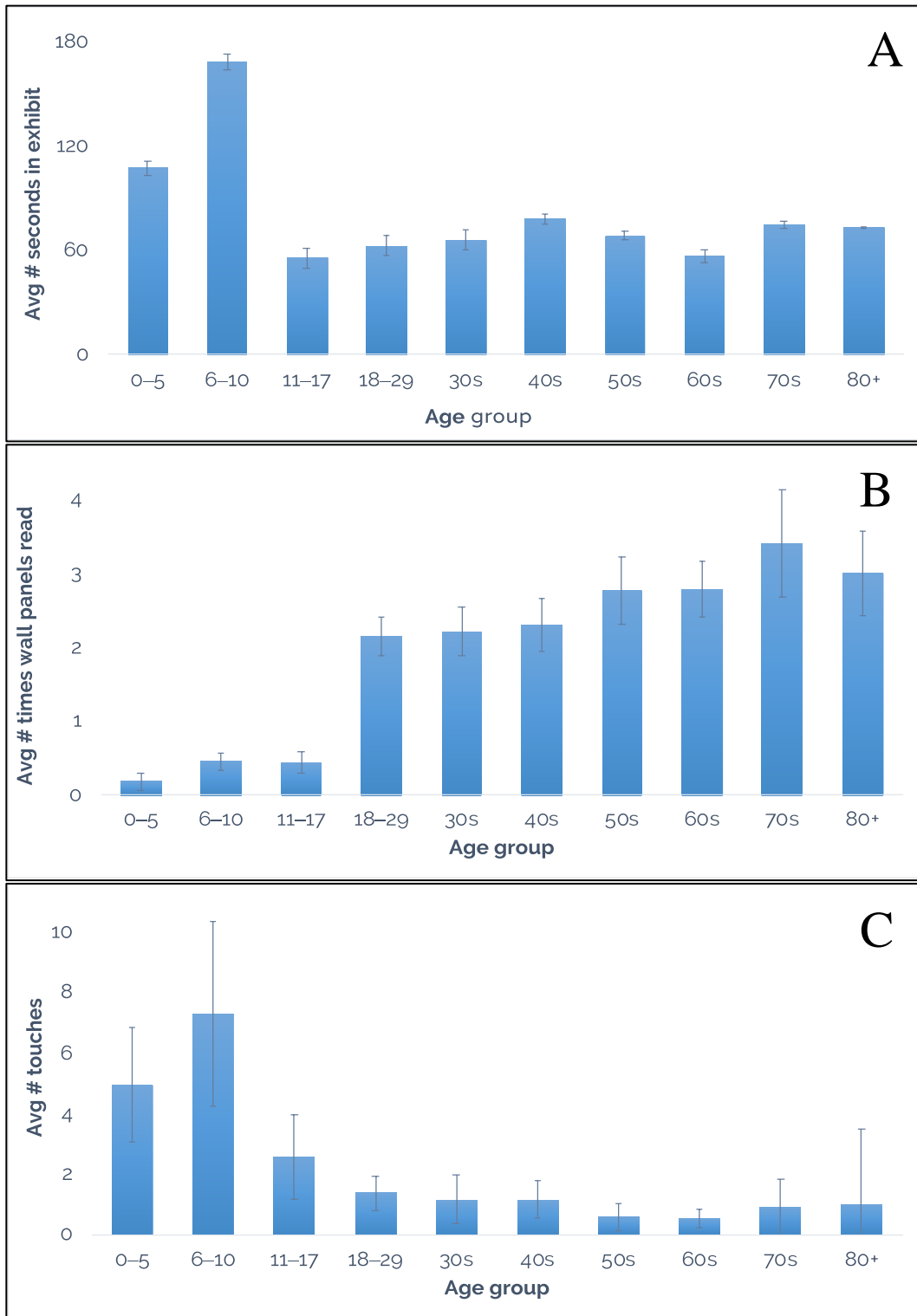
A total of 317 timed interactions were recorded in August (n= 170) and November 2019 (n= 147), and included females (n= 169) and males (n= 148) in age groups ranging from 0–5 (youngest; n= 38), to 80+ years (oldest; n= 3). Wall panels were read 411 total times, sometimes multiple times per individual. All four panels were read roughly equivalently, with Panel 1 (*Our plastic problem*) garnering 105 total reads, Panel 4 (*It's Time for Solutions*) garnering 100 reads, Panel 3 (*Microplastics in Coastal Critters*) with 99 reads and Panel 2 (*Plastic in the Environment*) with 96 reads. If using total number of touches as a proxy for interest in the exhibit elements, the *Zoom in on Microplastics* stand garnered the most interest (n= 1,118 total touches), followed by *Can You Help Clean the Beach?* (n= 800 total touches) and the *Plastic Solutions Desk* (n= 383 total touches).

#### Time and interaction differences by age, gender, and crowding level

Children aged 6–10 spent, on average, significantly more time in the exhibit than all other age groups ( $168.56 \pm 4.33$  seconds;  $F_{9,306} = 6.302$ ,  $p < 0.0001$ ), with children aged 0–5 spending the second highest amount of time in the exhibit ( $107.63 \pm 4.03$  seconds; Figure 3A). There was not a significant gender difference in time spent in the exhibit, as females spent an average of  $93.75 \pm 7.98$  seconds and males spent  $81.19 \pm 7.80$  seconds ( $T = 1.050$ ,  $p = 0.295$ ). Visitors spent more, but not significantly more, time in the exhibit space in November (avg=  $96.48 \pm 7.05$  seconds), when the aquarium was less crowded,

than in August (avg= 80.44 ± 8.89 seconds; T= -1.41, p= 0.159). Similarly, visitors spent more (but not significantly more) time in the exhibit space on Mondays (avg= 109.75 ± 15.51 seconds) than other survey days (Thursdays, Fridays, Saturdays, Sundays; avg= 87.88 ± 5.60 seconds;  $F_{4,312} = 1.070$ , p= 0.372).

When interaction types (reading, touching) were analyzed by age group (Figure 3C) and quantified by the number of times any interactive components were touched, visitors in the two oldest age groups (70s or 80+) read wall panels more frequently than all other age groups ( $F_{9,307} = 14.790$ , p< 0.0001; Figure 3B), and visitors in the two youngest age groups (0–5 and 6–10) touched the three interactive exhibit elements more frequently than all other age groups ( $F_{9,307} = 9.925$ , p< 0.0001; Figure 3C). Females interacted with the *Can You Help Clean the Beach?* table marginally more than males (females: avg= 3.11 ± 0.63, n= 523 touches; males: avg=1.91 ± 0.35, n= 277 touches; T= 1.678, p= 0.094). Average number of *Zoom in on microplastics* table interactions did not differ by gender (females: avg= 3.83 ± 0.51, n= 636 touches; males: avg= 3.44 ± 0.73, n= 482 touches; T= 0.553, p= 0.580), nor did interactions with the *Plastic Solutions Desk* (females: avg= 1.26 ± 0.18, n= 212 touches; males: avg= 1.16 ± 0.18, n= 171 touches; T= 0.321, p= 0.749).



**FIGURE 3 (A–C):** By age group, average A) number of seconds spent in the ocean plastic exhibit, B) number of times ocean plastic exhibit wall panels were read, and C) number of times ocean plastic exhibit interactive podia were touched.

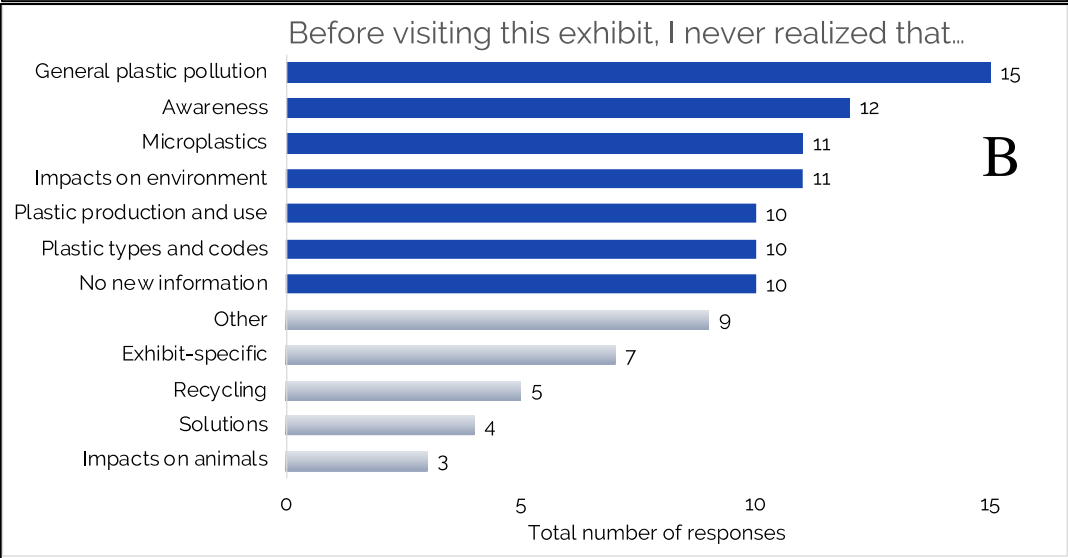
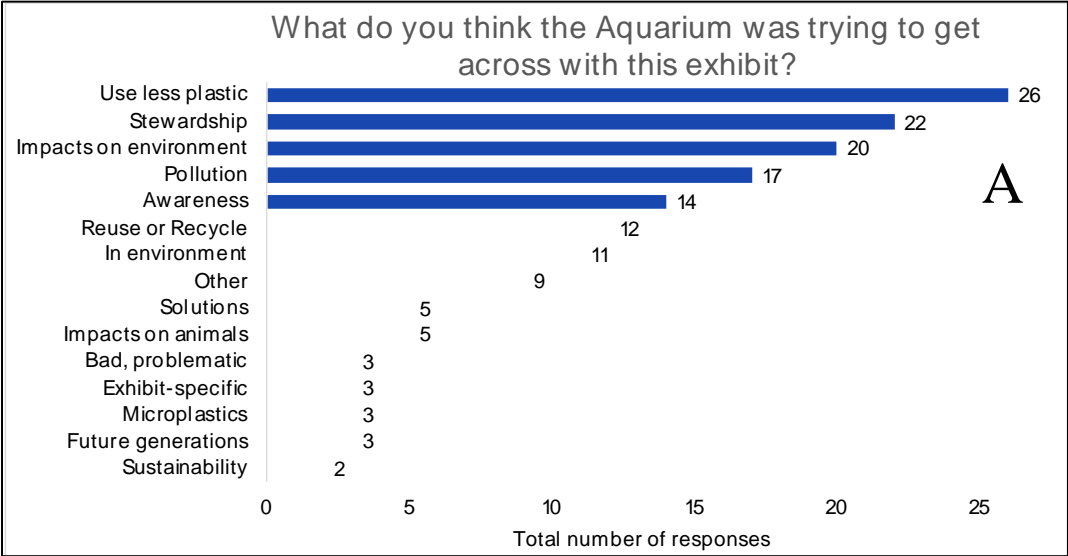
### *Structured Questionnaire*

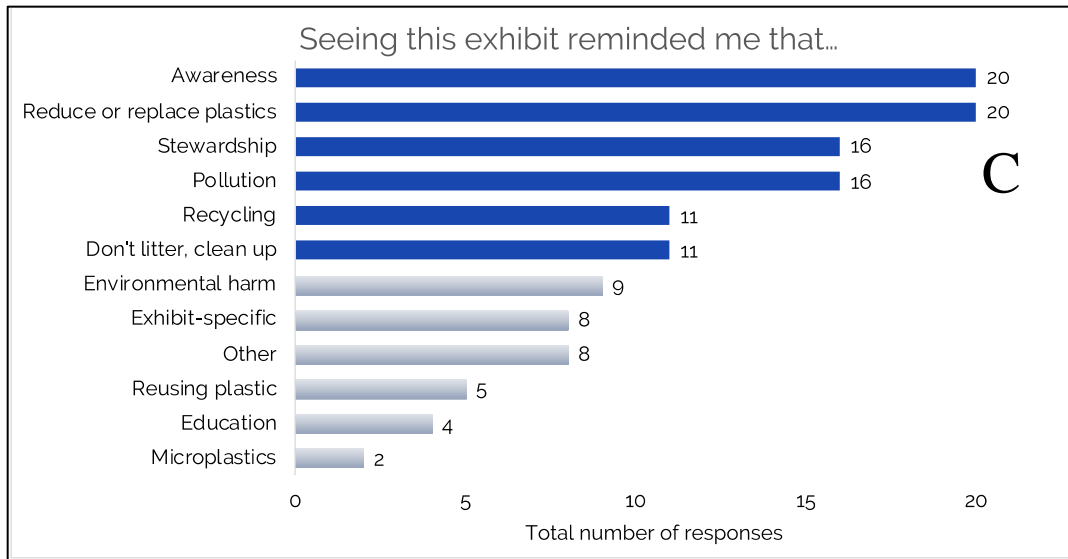
Structured questionnaires with mixed question types were administered to 93 randomly-selected visitors who engaged with the ocean plastic exhibit (Appendix N: Chapter 4). Our survey group included both females (n= 50) and males (n= 43), aged 18–29 (youngest), to 70–79 years old (oldest), representing 74 unique zip codes from 12 U.S. states (Arizona, California, Hawaii, Idaho, Kentucky, Nevada, Oklahoma, Oregon, Texas, Utah, Washington, Wisconsin) and two countries (U.S., Italy). The majority of respondents (64.5%; n= 60) were repeat OCA visitors. Seventy-one percent of respondents were at OCA with family or friends including children younger than 18 (n= 66), while others came with family or friends over the age of 18 (n=22), by themselves (n=3), or with organized groups (n= 2). Most respondents (87.1%; n=81) were first-time visitors to the ocean plastic exhibit.

### Communicating key messages

To determine if visitors understood key messages from the ocean plastic exhibit, we asked, “What do you think the Aquarium was trying to get across to you with this exhibit?” (Question 3, Appendix N: Chapter 4). Responses were thematically categorized, and the top three themes that emerged were: 1) use less plastic, 2) environmental stewardship, and 3) impacts on the environment (Figure 4A). Example responses from each of these categories were, “Start using less plastic, pay more attention. Restoration is important”, “Keeping the beaches clean, encouraging kids to take a closer look at plastics”, and “The impact of plastic on the environment and sea life”.

To determine what specific information presented in the exhibit was new to visitors, we asked, “Before seeing this exhibit, I never realized that...” (Question 5, Appendix N: Chapter 4). The top three response themes that emerged were: 1) general plastic pollution, 2) awareness, and 3) microplastics (Figure 4B). Examples of responses from each of these categories include: “How big the plastic problem is. Eight million tons dumped every year!”, “How many things have plastic in them”, and “I have been hearing a lot about microfibers but when I see them, I see why what comes off of my clothes ...impacts on the environment”. To determine what information presented was familiar to visitors, we asked, “Seeing this exhibit reminded me that...” (Question 6, Appendix N: Chapter 4). The top three themes that arose were: 1) awareness, 2) reducing or replacing plastics, and 3) environmental stewardship (Figure 4C). Examples of responses from each of these categories were: “We should be mindful of what we throw away and use every day”, “I can do better on not using single-use plastics”, and “Choices we make have long-lasting influence on the environment”.





**FIGURE 4 (A–C):** Thematically coded open-ended responses by 93 Oregon Coast Aquarium visitors to the questions- A) “What do you think the Aquarium was trying to get across to you with this exhibit?”; B) “Before seeing this exhibit, I never realized that...”; and C) “Seeing this exhibit reminded me that...”. Top five responses by frequency are represented in blue, remaining responses are represented in gray. Not all respondents provided answers for both questions. Non-responses were excluded from the figure.

We asked respondents to provide two positive words that came to mind when they heard the word ‘plastic’, followed by two negative words (Questions 13 and 14, Appendix N: Chapter 4). The top three themes for positive words were: 1) convenience and usefulness, 2) durability, and 3) cheap (Appendix R: Chapter 4), and the top themes for negative words were: 1) pollution and litter, 2) long-lasting, and 3) harm to environment or animals (Appendix R: Chapter 4).

#### Desire to help address the plastic pollution problem

Respondents (n= 91) self-reported their desire to help address the ocean plastic problem before their aquarium visit and after seeing the exhibit (Questions 11A and 11B, Appendix N: Chapter 4). Rankings of desire to help were from 0–4 (0= low, 4= high). Desire rankings were analyzed by gender, age, coastal status, and locality (Table 2).

Coastal status and locality were generated from zip codes provided. Coastal status was split by states with and without marine coastlines; locality was split between Oregonians and non-Oregonians. Overall, desire to help address the ocean plastic problem increased an average of 0.5 points from 3.2 to 3.7 after seeing the exhibit (Table 2). The largest change in desire was by 18–29 year olds (avg= 2.5 before, 3.5 after; Table 2).

**TABLE 2.** Mean responses for 91 Oregon Coast Aquarium visitors who self-reported their desire to help address the ocean plastic problem before and after seeing the ocean plastic exhibit. Rankings of desire to help were from 0–4 (0 = low, 4 = high). 87 respondents provided zip codes. U.S. states with marine coastlines were CA, HI, OR, TX, and WA; U.S. states with no marine coastlines were AZ, ID, KY, NV, OK, UT and WI. The single respondent with a non-U.S. address (Italy) was excluded from the locality and coastal categories.

	Respondent group	Number of respondents	Before seeing exhibit Average (SE)	After seeing Exhibit Average (SE)	Increase in desire to help address ocean plastic problem after seeing exhibit
<b>All</b>	<b>All respondents</b>	<b>91</b>	<b>3.2 (0.1)</b>	<b>3.7 (0.1)</b>	<b>0.5</b>
<b>Gender</b>	Females only	49	3.2 (0.1)	3.8 (0.1)	<b>0.6</b>
	Males only	42	3.2 (0.1)	3.6 (0.1)	<b>0.4</b>
<b>Age</b>	18-29 only	12	2.5 (0.4)	3.5 (0.2)	<b>1.0</b>
	30-39 only	25	3.2 (0.2)	3.7 (0.1)	<b>0.5</b>
	40-49 only	29	3.4 (0.1)	3.8 (0.1)	<b>0.4</b>
	50-59 only	11	3.5 (0.2)	4.0 (0.0)	<b>0.5</b>
	60-69 only	9	3.6 (0.2)	3.8 (0.1)	<b>0.2</b>
	70-79 only	4	3.0 (0.7)	3.0 (0.7)	<b>0.0</b>
<b>Coastal</b>	States with marine coastlines	74	3.3 (0.1)	3.7 (0.1)	<b>0.4</b>
	States with no marine coastline	11	3.3 (0.3)	3.7 (0.2)	<b>0.4</b>
<b>Locality</b>	Oregon zipcodes only	55	3.3 (0.1)	3.8 (0.1)	<b>0.5</b>
	Non-Oregon zipcodes only	30	3.1 (0.2)	3.6 (0.1)	<b>0.5</b>

Note: SE= Standard Error



### Single-use plastic reduction or plastic stewardship actions and intent to change

Single-use plastic reduction and plastic stewardship actions were self-reported by 91 OCA visitors (Question 4, Appendix N: Chapter 4) who were asked how often they participated in 10 single-use plastic reduction or plastic stewardship actions (1-carry a reusable water bottle, 2-bring your own coffee container, 3-bring your own bag to store or market, 4-choose items not packaged in plastic, 5-specifically ask for no straw, 6-use a reusable straw, 7-clean the beach, 8-carry your own utensils, 9-contact companies or representatives, 10-bring your own containers to restaurants) in their daily lives (Question 4, Appendix N: Chapter 4).

The single-use plastic reduction or plastic stewardship actions with the highest mean value reported were: 1) carry a reusable water bottle, 2) bring your own coffee container, and 3) bring your own bag to the store or market (Table 3). When comparing genders, females (n= 50) had higher mean scores than males (n= 41) for 7 of 10 actions (Table 3). The youngest age group sampled, 18–29 (n= 12), reported the lowest mean scores of all age groups for 7 of 10 actions and were second lowest for the remaining 3 actions (specifically ask for no straw, clean the beach, and carry your own utensils; Table 3). Respondents from states with marine coastlines (n= 76) reported higher mean responses to all actions except bringing their own containers to restaurants, in which case respondents from inland states (n= 11) had a higher mean score (Table 3). Mean action scores were not markedly different between Oregonians (n= 56) and non-Oregonians (n= 31), as Oregonians reported higher mean action scores for 5 actions, tied with non-

Oregonians for 1 action, and were outranked by non-Oregonians for the remaining 4 actions (Table 3).

**TABLE 3.** Mean responses for 91 questionnaire respondents who self-reported single-use plastic or plastic stewardship actions (prior to seeing exhibit). Actions are sorted left to right from highest to lowest mean response for all respondents. Responses are divided by gender, age, coastal status, and locality. 0= Never; 1= Rarely (I have done this a couple of times); 2= Sometimes (about once or twice a week); 3= Frequently (three or more days a week); 4= Almost every day. NAs and non-responses were excluded. There were no responses for the 80+ category. Only 87 respondents provided zip codes. U.S. states with marine coastlines were CA, HI, OR, TX, and WA; U.S. states with no marine coastlines were AZ, ID, KY, NV, OK, UT and WI. The single respondent with a non-U.S. address (Italy) was excluded from the locality and coastal categories.

	Respondent group	Number of respondents	Carry a reusable water bottle	BYO coffee container	BYO bag to store or market	Choose items not packaged in plastic	Specifically ask for no straw	Use a reusable straw	Clean the beach	Carry your own utensils	Contact companies or representatives	BYO containers to restaurant
All	All respondents	91	3.4	2.6	2.5	2.1	1.7	1.6	1.5	1.0	0.4	0.4
Gender	Females only	50	3.6	2.7	2.6	2.2	1.8	1.6	1.6	0.9	0.4	0.3
	Males only	41	3.3	2.6	2.4	1.9	1.7	1.5	1.4	1.0	0.5	0.4
Age	18-29 only	12	3.3	1.7	1.1	1.5	1.2	0.7	0.8	0.7	0.0	0.0
	30-39 only	26	3.4	2.6	2.4	2.3	1.9	2.0	1.7	1.0	0.2	0.3
	40-49 only	30	3.7	2.8	3.0	2.1	1.9	1.6	1.7	1.2	0.5	0.7
	50-59 only	11	3.7	3.2	2.5	2.6	1.9	1.9	1.9	0.9	1.0	0.2
	60-69 only	9	2.7	2.5	2.9	1.6	1.4	1.0	0.5	1.1	0.4	0.1
	70-79 only	3	3.3	4.0	4.0	1.7	1.0	1.0	2.3	0.0	0.3	0.0
Coastal	States with marine coastlines	76	3.5	2.7	2.6	2.1	1.7	1.5	1.6	1.1	0.4	0.3
	States with no marine coastline	11	3.3	2.4	2.0	1.8	1.6	1.4	1.0	0.5	0.3	0.5
Locality	Oregon zipcodes only	56	3.5	2.8	2.5	2.1	1.6	1.6	1.4	1.2	0.3	0.2
	Non-Oregon zipcodes only	31	3.4	2.4	2.5	2.0	1.9	1.5	1.8	0.7	0.6	0.6

Note: BYO= Bring your own.

Actions with the highest percentage of respondents reporting intended change were for using a reusable straw (37.4%), cleaning the beach (37.4%), bring-your-own (BYO) bag to the store or market (33/0%), and carrying your own utensils (30.8%; Table 4). Percent of respondents reporting intended change for other actions ranged from 11.0%–27.5% (Table 4). All age groups (18–29, 30–69, 70+) reported at least some intended changes for single-use plastic reduction and plastic stewardship actions. The 18–29 and 30–69 groups reported intended change  $\geq 14.9\%$  for all 10 actions, whereas the 70–79 year old group reported intended change for only 7 of 10 actions, with no intended

changes to carrying a reusable water bottle, carrying own utensils or BYO containers to restaurants (Table 4). When respondents were divided into those residing in states with (n=76) and without (n=11) marine coastlines, those residing in states with no marine coastline reported the highest percent of intended change for BYO coffee containers (54.5%) and specifically ask for no straw (54.5%). In most cases, the percentage of respondents reporting probable change was greater than or equal to the percentage reporting definite change, except there was a high proportion of respondent groups for which definite change was greater than or equal to the percentage reporting probable change for the carry a reusable water bottle action (Table 4). Oregon residents were more likely to change some plastic use actions than non-residents, and younger (18–50s) respondents were more likely to change their actions than older (60s–80+) respondents (Table 4).

**TABLE 4.** Percentage of Oregon Coast Aquarium visitors that self-reported **intended** single-use plastic or plastic-related environmental stewardship action changes after seeing the exhibit. Per respondent group, intended change is reported as: total percentage (number of individuals in the group reporting either probable –“probably will start doing or increase”, or definite - “definitely will start doing or increase” behavior change); percentage reporting probable change, and percentage reporting definite change. Groups and actions reporting ≥40% total change are colored green. Groups and actions where % reporting definite change > % reporting probable change are colored blue. Age was divided into three groups based on similarity of responses. Only 87 respondents provided zip codes. U.S. states with marine coastlines were CA, HI, OR, TX, and WA; U.S. states with no marine coastlines were AZ, ID, KY, NV, OK, UT and WI. The single respondent with a non-U.S. address (Italy) was excluded from the locality and coastal categories.

	Respondent group (n-)	Percentage of change	Carry a reusable water bottle	BYO coffee container	BYO bag to store or market	Choose items not packaged in plastic	Specifically ask for no straw	Use a reusable straw	Clean the beach	Carry your own utensils	Contact companies or representatives	BYO containers to restaurant
All	All (n=91)	% reporting change (total)	<b>11.0%</b>	<b>27.5%</b>	<b>33.0%</b>	<b>15.4%</b>	<b>28.6%</b>	<b>37.4%</b>	<b>37.4%</b>	<b>30.8%</b>	<b>24.2%</b>	<b>25.3%</b>
		% reporting probable change	4.4%	15.4%	24.2%	9.9%	18.7%	20.9%	26.4%	20.9%	18.7%	22.0%
		% reporting definite change	6.6%	12.1%	8.8%	5.5%	9.9%	16.5%	11.0%	9.9%	5.5%	3.3%
Gender	Females only (n=50)	% reporting change (total)	<b>10.0%</b>	<b>32.0%</b>	<b>40.0%</b>	<b>12.0%</b>	<b>22.0%</b>	<b>36.0%</b>	<b>42.0%</b>	<b>32.0%</b>	<b>26.0%</b>	<b>30.0%</b>
		% reporting probable change	4.0%	16.0%	26.0%	6.0%	14.0%	22.0%	32.0%	20.0%	20.0%	24.0%
		% reporting definite change	6.0%	16.0%	14.0%	6.0%	8.0%	14.0%	10.0%	12.0%	6.0%	6.0%
	Males only (n=41)	% reporting change (total)	<b>12.2%</b>	<b>22.0%</b>	<b>24.4%</b>	<b>19.5%</b>	<b>36.6%</b>	<b>39.0%</b>	<b>31.7%</b>	<b>29.3%</b>	<b>22.0%</b>	<b>19.5%</b>
		% reporting probable change	4.9%	14.6%	22.0%	14.6%	24.4%	19.5%	19.5%	22.0%	17.1%	19.5%
		% reporting definite change	7.3%	7.3%	2.4%	4.9%	12.2%	19.5%	12.2%	7.3%	4.9%	0.0%
Age	18-29 only (n=12)	% reporting change (total)	<b>25.0%</b>	<b>16.7%</b>	<b>33.3%</b>	<b>16.7%</b>	<b>33.3%</b>	<b>41.7%</b>	<b>25.0%</b>	<b>41.7%</b>	<b>25.0%</b>	<b>25.0%</b>
		% reporting probable change	0.0%	8.3%	16.7%	0.0%	8.3%	33.3%	16.7%	33.3%	25.0%	16.7%
		% reporting definite change	25.0%	8.3%	16.7%	16.7%	25.0%	8.3%	8.3%	8.3%	0.0%	8.3%
	30-59 only (n=74)	% reporting change (total)	<b>9.5%</b>	<b>14.9%</b>	<b>25.7%</b>	<b>31.1%</b>	<b>36.5%</b>	<b>29.7%</b>	<b>23.0%</b>	<b>31.1%</b>	<b>25.7%</b>	<b>41.9%</b>
		% reporting probable change	5.4%	9.5%	14.9%	21.6%	21.6%	20.3%	17.6%	20.3%	21.6%	29.7%
		% reporting definite change	4.1%	5.4%	10.8%	9.5%	14.9%	9.5%	5.4%	10.8%	4.1%	12.2%
	70-79 only (n=4)	% reporting change (total)	<b>0.0%</b>	<b>25.0%</b>	<b>50.0%</b>	<b>25.0%</b>	<b>50.0%</b>	<b>50.0%</b>	<b>50.0%</b>	<b>0.0%</b>	<b>25.0%</b>	<b>0.0%</b>
		% reporting probable change	0.0%	25.0%	25.0%	25.0%	25.0%	50.0%	50.0%	0.0%	25.0%	0.0%
		% reporting definite change	0.0%	0.0%	25.0%	0.0%	25.0%	0.0%	0.0%	0.0%	0.0%	0.0%
Coastal	States with marine coastlines (n=76)	% reporting change (total)	<b>10.5%</b>	<b>23.7%</b>	<b>34.2%</b>	<b>17.1%</b>	<b>23.7%</b>	<b>36.8%</b>	<b>39.5%</b>	<b>31.6%</b>	<b>22.4%</b>	<b>26.3%</b>
		% reporting probable change	2.6%	13.2%	26.3%	11.8%	17.1%	21.1%	28.9%	23.7%	17.1%	25.0%
		% reporting definite change	7.9%	10.5%	7.9%	5.3%	6.6%	15.8%	10.5%	7.9%	5.3%	1.3%
	States with no marine coastline (n=11)	% reporting change (total)	<b>9.1%</b>	<b>54.5%</b>	<b>18.2%</b>	<b>9.1%</b>	<b>54.5%</b>	<b>45.5%</b>	<b>27.3%</b>	<b>18.2%</b>	<b>45.5%</b>	<b>18.2%</b>
		% reporting probable change	9.1%	36.4%	18.2%	0.0%	27.3%	27.3%	18.2%	0.0%	36.4%	9.1%
		% reporting definite change	0.0%	18.2%	0.0%	9.1%	27.3%	18.2%	9.1%	18.2%	9.1%	9.1%
Locality	Oregon zipcodes only (n=56)	% reporting change (total)	<b>10.7%</b>	<b>23.2%</b>	<b>33.9%</b>	<b>17.9%</b>	<b>21.4%</b>	<b>37.5%</b>	<b>41.1%</b>	<b>26.8%</b>	<b>23.2%</b>	<b>25.0%</b>
		% reporting probable change	1.8%	12.5%	23.2%	10.7%	16.1%	23.2%	28.6%	21.4%	17.9%	25.0%
		% reporting definite change	8.9%	10.7%	10.7%	7.1%	5.4%	14.3%	12.5%	5.4%	5.4%	0.0%
	Non-Oregon zipcodes only (n=31)	% reporting change (total)	<b>9.7%</b>	<b>35.5%</b>	<b>29.0%</b>	<b>12.9%</b>	<b>38.7%</b>	<b>38.7%</b>	<b>32.3%</b>	<b>35.5%</b>	<b>29.0%</b>	<b>25.8%</b>
		% reporting probable change	6.5%	22.6%	29.0%	9.7%	22.6%	19.4%	25.8%	19.4%	22.6%	19.4%
		% reporting definite change	3.2%	12.9%	0.0%	3.2%	16.1%	19.4%	6.5%	16.1%	6.5%	6.5%

Note: BYO= Bring your own.

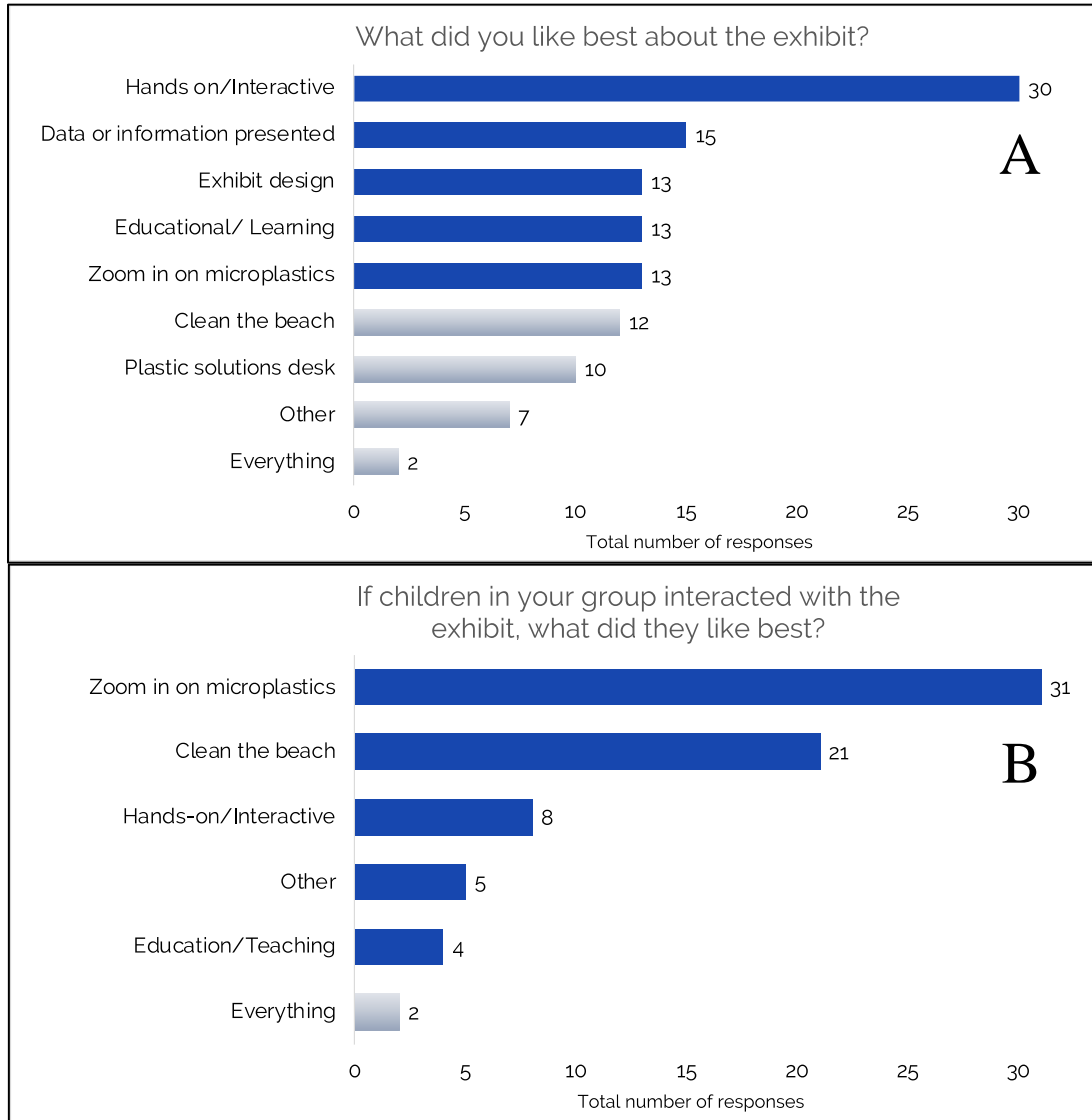
### Impressions & Learning

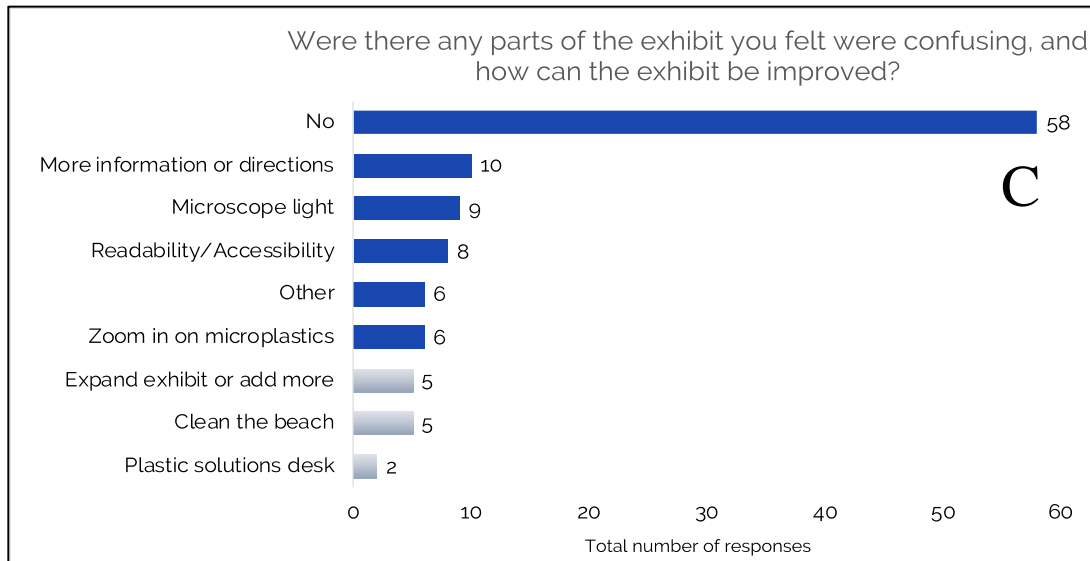
Overall, the ocean plastic exhibit left very positive impressions on respondents. When asked, “How would you rate your overall impression of this exhibit?” on a scale of 0–4, (0 being low and 4 being high), the average score was 3.60 (n=93; range= 2–4), with 96.7% (n= 88) rating the exhibit either a 3 or 4 (Question 15, Appendix N: Chapter 4), and 62.6% (n= 57) giving the highest possible mark of a 4. When asked, “On a scale of ‘Strongly disagree’ to ‘Strongly agree’: I learned from interacting with this exhibit” (Question 8, Appendix N: Chapter 4), 50% of respondents (n = 46) agreed, 43.5% (n= 40) strongly agreed, 5.4% (n= 5) were neutral, and 1.1% (n= 1) strongly disagreed. Roughly 70% (n= 65) of respondents reported coming to the Aquarium with a child or children (age range of respondent-associated children= 10 months–17 years); of those 65 individuals who were asked: “Please answer this statement on a scale of ‘Strongly disagree’ to ‘Strongly agree’: My child learned from interacting with this exhibit” (Question 9B, Appendix N: Chapter 4), 46.2% (n= 30) agreed, 35.4% (n= 23) strongly agreed, 16.9% (n= 11) were neutral, 1.5% (n= 1) disagreed, and 0% (n= 0) strongly disagreed.

### Most liked exhibit elements and suggested areas for improvement

When asked “What did you like best about the exhibit”, most respondents (n=30) liked the interactive, hands-on nature of the exhibit best (Figure 5A; Question 9A- Appendix N: Chapter 4). The information presented, design of the exhibit, learning opportunities afforded, and the *Zoom in on microplastics* stand element in particular, were also well-liked (Figure 5A). Children of the respondents liked the *Zoom in on*

*microplastics* stand best (43.7%; n=31), followed by the *Can You Help Clean the Beach?* stand (29.6%; n= 21; Figure 5B).





**FIGURE 5.** (A-C): Thematically categorized open-ended responses by 93 Oregon Coast Aquarium visitors for the questions: A) “What did you like best about the exhibit”; B) “If children in your group interacted with the exhibit, what did they like best?”; and C) “Were there any parts of the exhibit you felt were confusing and how could the exhibit be improved?”. Top five responses by frequency are represented with blue bars, remaining responses are represented with gray bars. Non-responses due to no children in the group were not included in Figure 5B. Note: the microscope light did not work during the August sampling period.

When asked, “Were there any parts of the exhibit you felt were confusing, and how could the exhibit be improved?”, most respondents (53.2%; n= 58) said ‘no’ (Figure 5C; Question 10, Appendix N: Chapter 4). The most common areas identified for potential exhibit improvement were adding more information or directions to the interactive elements and adjusting/fixing the microscope light (Figure 5C). The microscope light malfunctioned during the August data collection period, but was repaired for the November data collection period.

#### 4.4 Discussion and Conclusions:

Using education to promote shifts in individual behaviors or actions that reduce single-use plastic is one tool among many that must be employed to meaningfully reduce ocean plastic pollution going forward. Emphases must also be placed further upstream at the production level, well before plastic products reach market shelves or consumer hands, so that unnecessary and dangerous single-use products are no longer produced en masse and are prevented from reaching coasts and oceans. We created an exhibit at the Oregon Coast Aquarium to illustrate the problem of ocean plastic, focusing on conservation messaging that offered visitors an opportunity to contribute to a suite of solutions. Our findings illuminate strategies for future ocean plastic exhibits to effectively reach broad audiences, including specific single-use plastic reduction and plastic stewardship actions that could be effective targets for education and outreach.

As expected, Children (0–17 years) rarely looked at the informational panels but gravitated toward the interactive exhibit elements; this could be partially due to the fact that young children that cannot read may naturally be attracted to interactive features rather than visuals (Ross and Gillespie 2008). There were no significant differences in total time spent in the exhibit when comparing peak (August) and off-season (November) timeframes, or day of the week; this was a surprising result, as dense crowds around exhibits may influence time spent in an exhibit by either discouraging or encouraging interest (Yalowitz and Bronnenkant 2009). Crowds within the exhibit were visibly larger in August than November, and on weekends than weekdays.



While respondents' intentions to change single-use plastic stewardship actions were evident, for a number of respondent groups there was greater than a 10% difference between probable and definite changes to their actions (Table 4). These differences should be considered for targeted outreach to respective respondent groups to further encourage behavioral change. Up to 37.4% of respondents were receptive to changing some actions—namely using reusable straws, cleaning the beach, carrying your own utensils and BYO bag to the store or market. If people report that they want to try new behaviors, promoting these behaviors more broadly may be more successful since people are receptive to making self-reported changes. Providing simple, clear-cut guidance on ways to effectively implement those changes in daily life may also facilitate adoption of those actions. Conversely, respondents were unlikely to propose changing how frequently they carry a reusable water bottle, specifically choose items not packaged in plastic, contact companies or representatives, and BYO containers to a restaurant. It is likely the small intent to change carrying a reusable water bottle was because that behavior had already been widely adopted by respondents prior to seeing the exhibit (Appendix S: Chapter 4); however, for the other three, providing carefully-crafted and widely available information on the ways these actions reduce ocean plastic, and distributing guidance regarding simple techniques for adopting these actions could help move the dial on household single-use plastic reduction.

To date, environmental behaviors have largely been quantified through self-reporting due to low cost and ease of data collection (Pahl and Wyles 2017). Self-reported behaviors can be used to gauge behavior in real-life scenarios, though they are

not perfect reflections of reality (Bamberg and Möser 2007; Kormos and Gifford 2014; Pahl and Wyles 2017). As with other behavioral studies, including those where individuals self-report intended behavioral changes, our study has inherent limitations. The extent to which aquarium visitors learn from exhibits and subsequently change behaviors can depend on outside factors including their motivations for visiting the aquarium, pre-existing perceptions, knowledge, interests, and personal experiences (Moussouri 1997; Falk and Adelman 2003; Storksdieck 2006). We did not ground truth whether intended single-use plastic reduction or plastic stewardship actions resulted in actual behavioral changes after interacting with the ocean plastic exhibit.

Of all age groups in our study, 18–29 year olds had the highest change in desire to help address the ocean plastic problem before and after seeing the exhibit (Table 2), but the lowest mean scores for most of the 10 single-use plastic and stewardship actions examined (Table 3). The former could be because 18–29 year olds reported the lowest score of any age group for desire to help address the ocean plastic problem prior to seeing the exhibit and thus had the most room for growth of any age group post-exhibit. Though this group reported a strong desire to decrease contributions to ocean plastic, they are evidently failing to take actions to make that happen. This suggests a mismatch between their awareness of, or sense of personal responsibility for, the ocean plastic problem and concrete plastic-reduction or stewardship actions—a trend that has been previously identified for this generation (Heidbreder et al. 2019). This may be because some groups don't see the plastic problem as an individual problem and instead view it as a societal problem. This can result in a failure to take action (Nalven 2019). Further cultivating a

civic responsibility mindset for this age group with specific educational or outreach goals and remedial actions should be explored to close the awareness-to-action gap, leading to long-lasting behavioral changes and positive environmental outcomes.

Future work monitoring durable changes to single-use plastic or plastic-related environmental stewardship actions after educational experiences or interventions is needed to ground truth behavioral outcomes. Long-term behavioral changes are of immense importance given the longevity of plastics in the environment and need for sustained, permanent changes to individual plastic consumption as a means to improve environmental health. Reinforcing plastic reduction messaging over time for all age levels can aid in driving behavioral shifts that reduce single-use plastic dependency, ultimately leading to long-lasting positive change.

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## Chapter 5: Conclusions

A growing body of literature characterizes microplastics (MPs) as harmful to the organisms and ecosystems they pervade (Barnes et al. 2009; Lusher et al. 2017).

The research contained in Chapters 2 and 3 of this dissertation greatly improves our scientific understanding of MP prevalence in two prized Pacific Northwest (PNW) seafood species, the Pacific oyster (*Crassostrea gigas*) and Pacific razor clam (*Siliqua patula*). Our research confirms MPs are present in Pacific oysters and Pacific razor clams at all 23 sites sampled in Oregon (OR) and Washington (WA). It highlights the prevalence of MPs in the coastal environment, even in an area of relatively low human impact like the Olympic Coast, WA. Prior to this work, data had not been collected on MPs in Pacific razor clams anywhere in the world, and data on MP burdens in OR Pacific oysters were also absent.

We found OR Pacific oysters contained more MPs per individual and per gram of tissue than OR Pacific razor clams at our 15 study sites. In Pacific oysters purchased from six OR aquaculture growers, season influenced MP concentrations in Pacific oysters more than geography, body size or the anatomical differentiation between gut and tissue, with spring-collected individuals containing more MPs than summer-collected individuals and oysters containing more MPs than razor clams (Chapter 2; Baechler et al. 2020b). While not directly investigated, we posited that seasonal and species-specific MP differences may have been attributed to habitat (oysters inhabit estuarine environments, which receive stormwater runoff and wastewater inputs before the open coast-dwelling razor clams do; therefore, MPs may be more concentrated in estuaries), seasonal

precipitation patterns (higher precipitation in spring than summer, resulting in higher levels of MP-laden runoff reaching coastal organisms), or the type of clothing laundered in the spring vs summer (bulkier synthetics used and laundered in the spring due to colder temperatures). A few OR site-specific differences in MPs were detected in oysters but not razor clam samples.

Investigations of Olympic Coast, WA Pacific razor clams allowed us to determine MP burdens were lower per individual (MP/individual) than those in OR razor clams but were similar when comparing MP/g<sup>-1</sup> tissue between the two states (Chapter 3).

Polyethylene Terephthalate (PET), one of the most widely produced polymers in the world (33 million metric tons produced in 2015; Geyer et al. 2017) and commonly used in beverage containers and textiles, was the most frequently identified material in the subset of MPs chemically examined through Fourier Transform Infrared (FTIR) in both our OR and WA shellfish samples. The PET microfibers isolated from PNW shellfish samples investigated in Chapters 2 and 3 could have originated from numerous land-based sources including stormwater runoff and wastewater treatment plant effluent, but could also be derived from fishing-related activities including lost or abandoned fishing gear or associated ropes or lines (Chapter 1, Figure 1). While resin type was able to be determined through the use of Fourier Transform Infrared (FTIR), the specific origin of PET and other materials found in Pacific oysters and Pacific razor clams analyzed through this work could not be determined.

MP presence in Pacific Northwest (PNW) coastal food webs raises concern about the potential for ecological harm at numerous trophic levels. MPs in marine species

harvested and sold for human consumption could pose threats to human health. While we do know that humans ingest MPs (through inhalation or direct consumption), our understanding of specific impacts to key human endpoints (growth, reproduction, cognitive abilities, fitness) are limited (Smith et al. 2018; Yong et al. 2020). Our estimates of MP exposure from razor clams by Olympic Coast harvester-consumers were 60–3,070 MP/yr for cleaned or 120–6,020 MP/yr for whole clams, illustrating the importance of cleaning clams prior to consumption to reduce human MP exposure. We identified significant differences in harvester-consumer MP exposure based on gender and ethnicity. Due to the low sample size of Alaska Native and Native American questionnaire respondents in Chapter 3, we are unable to broadly generalize about clam consumption frequencies of individuals within these groups; however, responses we obtained from these few individuals were striking considering the much higher minimum exposure level of MPs through razor clam consumption than other ethnicities. Owing to this finding and previous reports that seafood consumption tendencies vary by ethnicity (e.g., Sechena et al. 1999; Mahaffey et al. 2009; EPA 2014; Cisneros-Montemayor et al. 2016), additional PNW-specific research regarding seafood consumption and subsequent microplastic exposure for these and other high-seafood consumer demographics is important and necessary.

Reducing exposure is particularly important in the absence of established safety thresholds for human MP intake. Fortunately, Olympic Coast razor clams appear to be a minimal vector of MP exposure within the recreational razor clam harvester-consumer group studied when compared to other known exposure routes of MPs to humans (e.g.,



inhalation, deposition of microfibers during meals, consumption of table salt or drinking bottled water; Catarino et al. 2018; Cox et al. 2019; Zhang et al. 2020a).

We identified a knowledge gap in the group of WA razor clam harvesters studied, whereby most of these direct resource users in our study did not possess knowledge about MP contamination in food items, but nonetheless harbored concerns about the concept (Chapter 3). Educating the public, including fishers and direct resource users, through campaigns or passive outreach (e.g., through aquarium exhibits) could be an effective means to disseminate MP information to broad audiences and promote future plastic-related environmental stewardship actions.

The OR Coast Aquarium ocean plastic exhibit designed, installed and evaluated as an educational tool (Chapter 4), demonstrated information transfer to aquarium visitors and helped elicit self-reported intent to change single-use plastic behaviors by those visitors. Visitors reported learning from exhibit engagement and communicated willingness to change behaviors in their daily lives to reduce single-use plastic items. Nearly 40% of aquarium questionnaire respondents were receptive to changing some actions—namely using reusable straws, cleaning the beach, carrying utensils and bring-your-own (BYO) bag to the store or market. Providing simple, effective guidance on ways to effectively implement these changes in daily life may facilitate adoption of those actions, as visitors reported interest and intent to change these actions and may be on the cusp of doing so. Conversely, respondents were unlikely to propose changing how frequently they carry a reusable water bottle, specifically choose items not packaged in plastic, contact companies or representatives, and BYO containers to a restaurant.

Providing concise, engaging, and widely available information on how these actions can ultimately lead to a reduction in ocean plastic could move the dial on household single-use plastic reduction via employment of these actions.

The data generated in this dissertation on MP prevalence in shellfish, MP exposure through harvest and consumption of said shellfish, MP knowledge and concerns of recreational razor clammers and aquarium visitors, and single-use plastic behavioral change opportunities, has advanced the existing scientific knowledge base regarding the issue of marine plastics in the PNW. Results of this work have been disseminated to a variety of audiences throughout the PNW and more broadly through oral and poster presentations at scientific conferences, in classrooms and at informal pub events, through publications, installation of an educational aquarium exhibit, and via mainstream media coverage. I hope the array of techniques used to communicate these results will reach broad audiences in the PNW and beyond, inspiring current and future leaders to tackle the plastic pollution issue head-on in our region and around the world.

Ocean plastic pollution is an ever-growing issue that requires, among a suite of other interventions, education to inspire behavioral change and meaningfully reduce would-be future ocean plastic pollution. In addition to behavioral change, upstream reductions to plastic production, and improvements to better manage plastic waste and plastic breakdown products like MPs are critical to prevent pollution from reaching coasts and oceans (Chapter 1, Figure 2). This body of work highlights the issue of MP incorporation in two important PNW bivalve species, and also illuminates the ways in which Oregon Coast Aquarium visitors employ and are willing to change their single-use

plastic actions to reduce ocean plastic pollution. The results of my work contained in Chapters 2–4 can inform waste management, engineering solutions, and policy actions to reduce MP transmission into the ocean, and drive individual behavioral shifts that reduce single-use plastic dependency, ultimately leading to long-lasting positive change. Due to repeated findings of MPs in marine species worldwide, the need to overhaul our current microplastic management and mitigation practices is apparent. We must modify our extant technologies and behaviors appropriately to reduce future transmission of MPs, and in particular microfibers, into the environment. A focus on land-based solutions to MP pollution is imperative, as more than 80% of primary and secondary MPs originate on land (Miranda et al. 2019). The laundering of clothing is a significant vector of anthropogenic microfibers to the environment; since the 1950's, over 5.9 million metric tons of synthetic MPs have been generated through laundering (Gavigan et al. 2020). Our findings of primarily microfibers in PNW bivalves highlights a need for upstream microfiber pollution control at the source, such as integrating MP capture technologies into washing machines— a technology that will be required in France beginning in 2025 (European Parliament 2020). Broader adoption of similar technologies by the United States and other countries could drastically reduce global microfiber emissions to the environment moving forward.

This and similar works will be important references for future MP source reduction regulations, human toxicological risk assessments for the numerous MP categories, shapes, sizes, material types, and colors, and perhaps establishing human health standards, which are currently defined for many chemical contaminants but

unknown for MPs. Ultimately, it will be important for us as a society to not only prioritize research and impacts of MPs on marine species and humans, but to effect sustained behavioral changes and enact policies that meaningfully prevent these harmful pollutants from becoming further incorporated into food webs and the human diet. The actions we take today to abate further transmission of plastics into the environment will help preserve ocean health and protect the innumerable, indispensable resources and ecosystem services our global ocean provides.

## 5.1 References:

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## Appendices:

**APPENDIX A: CHAPTER 2.** Biological information for Pacific oysters and razor clams collected at each sample site in April (Spring) and July (Summer) 2017.

		Spring 2017			Summer 2017		
Spp.	Site name	Avg. shell length in mm (SE)	Avg. shell width in mm (SE)	Body tissue wet weight in g (SE)	Avg. shell length in mm (SE)	Avg. shell width in mm (SE)	Body tissue wet wt in g (SE)
Pacific oyster	OY1	111.75 (2.32)	60.56 (1.59)	26.96 (1.88)	112.21 (2.00)	66.68 (3.12)	24.10 (2.95)
	OY2	156.03 (5.86)	66.64 (2.48)	32.52 (1.82)	132.94 (4.43)	74.02 (4.43)	34.22 (2.49)
	OY3	121.77 (3.01)	65.55 (2.29)	24.06 (1.28)	133.18 (5.94)	68.44 (2.68)	32.76 (1.76)
	OY4	134.11 (3.76)	83.24 (4.36)	46.96 (3.54)	133.32 (3.30)	78.33 (3.29)	51.30 (6.23)
	OY5	119.79 (1.81)	62.87 (1.53)	19.15 (0.57)	100.56 (5.27)	51.49 (3.08)	19.12 (4.59)
	OY6	114.90 (2.27)	76.73 (1.98)	34.74 (2.36)	124.09 (6.36)	57.74 (2.78)	25.70 (2.21)
Pacific razor clam	Clatsop Beach	112.99 (2.12)	43.68 (1.20)	56.24 (3.50)	108.76 (1.51)	43.06 (0.46)	57.65 (2.02)
	Cannon Beach	118.89 (4.49)	46.61 (2.60)	55.60 (5.73)	110.34 (1.84)	43.30 (1.40)	59.22 (3.44)
	Cape Meares	112.65 (2.79)	45.24 (1.68)	49.45 (3.80)	84.81 (7.39)	32.88 (3.45)	26.24 (7.41)
	Agate Beach	N/A	N/A	N/A	121.41 (1.49)	48.83 (0.73)	68.72 (1.78)
	Newport S. Beach	114.78 (1.42)	48.12 (1.23)	45.91 (2.14)	120.20 (1.96)	49.41 (1.10)	71.44 (3.60)
	Coos Bay	115.83 (1.84)	48.14 (1.02)	40.84 (1.38)	N/A	N/A	N/A
	Bastendorff Beach	N/A	N/A	N/A	113.15 (4.36)	45.51 (1.74)	59.92 (4.36)
	Whiskey Creek	N/A	N/A	N/A	117.19 (1.47)	48.65 (1.14)	63.28 (4.43)
	Gold Beach	N/A	N/A	N/A	121.55 (1.51)	47.93 (0.69)	71.30 (3.00)

Notes: Spp. = Species; Avg. = Average; WT = Weight; SE = ± Standard Error; OY1-OY6: Oyster site (randomized).

**APPENDIX B: CHAPTER 2.** Sources, averages, and ranges of microplastic contamination in Pacific oyster and Pacific razor clam samples.

Sources of microplastic contamination	Description	Pacific oysters (Avg. per sample)	Pacific razor clams (Avg. per sample)	Range of microplastic counts per sample
Procedural controls	Filtered DI water run through chemical digestion, sieving, visual microscope analysis	4.91 ± 1.11	4.91 ± 1.11	0–10
Microscope blanks	Petri dish with filtered DI water open to the air during microscope analysis of regular samples.	0.20 ± 0.03	0.20 ± 0.03	0–5
Secondary digestion step (razor clams only)	Chemical digestion and hypersaline density separation	Not undertaken	1.00 microfiber per sample	1–1
<b>TOTAL Contamination</b>	Sum of all sources of contamination	<b>5.11</b>	<b>6.11</b>	N/A

Notes: Avg. = Average.

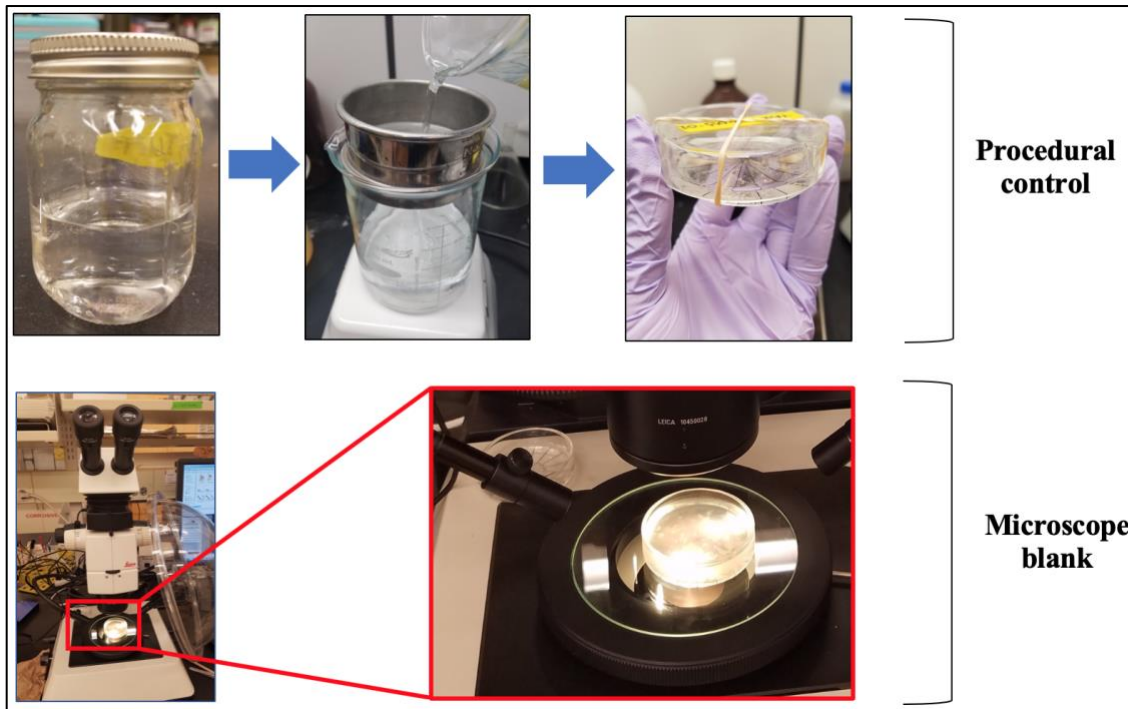


### Chapter 3

**APPENDIX C: CHAPTER 3.** 2010 Land use cover (km<sup>2</sup>) for the four coastal counties located on the Olympic Peninsula, WA. Counties are ordered north to south. Razor clam sample site names are listed below their respective county names. No razor clam sites were located in Clallam county. Data was obtained from NOAA's Digital Coast at: <https://coast.noaa.gov/digitalcoast/tools/lca.html>.

County	Land Use cover (sq. km)						
	Forested	Scrubland	Bare Land	Grassland	Wetland	Development	Agriculture
<b>Clallam county</b> (no sites)	3365	572	109	190	69	114	96
<b>Jefferson county</b> (Kalaloch beach)	3556	461	376	158	93	49	19
<b>Grays Harbor county</b> (Point Grenville, Mocrocks, Copalis beach, South Copalis, North Twin Harbor, South Twin Harbor)	2973	976	250	355	284	119	107
<b>Pacific county</b> (Long Beach)	1303	509	252	259	186	44	45

**APPENDIX D: CHAPTER 3.** Schematic of the two microplastic (MP) contamination quantification techniques employed in this study. Procedural controls were deionized water filtered to 50µm and run through the same process as all other samples. Microscope blanks were glass petri dishes filled with filtered DI water, placed on the microscope base, and left open to the air during visual inspection of samples for MPs.





Q5: Within the past year, when you consumed razor clams, you ate them:

- With guts
- Without guts
- Sometimes with guts, sometimes without
- I don't know

Q7: Within the past year, how did you obtain the razor clams you ate? Select all that apply.

- I dug them myself
- Friends or family gave them to me
- I purchased them
- I ate them at tribal events
- I traded or bartered for them
- Other (write in): \_\_\_\_\_
- I did not eat razor clams
- I don't know

Q6: Your favorite preparation of razor clams is:

- Soup, Stew, or Chowder
- Pan or deep fried
- Smoked
- Other (write in): \_\_\_\_\_

Q8: For each season within the past year, how often did you **collect** or **harvest** razor clams?

	Never	Once a month	Once a week	2-3 times a week	4-6 times a week	7+ times a week
Winter (Dec. to Feb.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Spring (March to May)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Summer (June to Aug.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Fall (Sept. to Nov.)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q9: Within the past year, roughly how often did you **eat** other marine shellfish species?

	Never	Less than 6 times a year	About 1 meal a month	2 or 3 meals a month	About 1 meal a week	2 to 4 meals a week	5 to 6 meals a week	7+ meals a week	I don't know
Oysters	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Mussels	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Bay Clams	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Hard shell clams	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Geoducks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dungeness Crab	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other crab (non-Dungeness)	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Shrimp	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Scallops	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Urchins	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Snails	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other shellfish (write in): _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q10: Within the past year, did you dig razor clams commercially (to sell)?

- Yes
- No

Q14: How old are you (in years)?

- 18-28
- 29-39
- 40-50
- 51-61
- 62+

Q11: How familiar are you with the concept of microplastics in food?

- Not familiar at all
- Moderately familiar
- Very familiar

Q15: What is your gender?

- Male
- Female
- Other

Q12: Do you have any concerns about microplastics in your food?

- Yes
- No
- I don't know

Q16: What is your ethnic identity? Select all that apply.

- Native American
- Alaska Native
- Native Hawaiian or Pacific Islander
- Black or African American
- Asian
- Latino
- White/Caucasian
- Other (write in): \_\_\_\_\_

Q13: Please expand on what concerns you have about microplastics in your food.

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Q17: What is/are your Tribal affiliation(s)? Select all that apply.

- Alsea
- Chinook
- Chehalis
- Clatskanie
- Coos
- Cowlitz
- Klallam
- Kwalhioqua
- Makah
- Quileute
- Quinault
- Siletz
- Siuslaw
- Tillamook
- Other (write in): \_\_\_\_\_
- No tribal affiliation

Q18: Which county do you live in?

**Washington**

- Clallam
- Clark
- Cowlitz
- Grays Harbor
- Jefferson
- Pacific
- Wahkiakum

**Oregon**

- Clatsop
- Coos
- Douglas
- Lane
- Lincoln
- Multnomah
- Tillamook

- Other (write in): \_\_\_\_\_
- I do not live in Washington or Oregon

Q21: What is your estimated income per year from all sources (in US dollars)?

- Less than \$15,000
- \$15,001-\$25,000
- \$25,001-\$35,000
- \$35,001-\$50,000
- \$50,001-\$75,000
- \$75,001-\$100,000
- \$100,001-\$150,000
- More than \$150,000

Q19: What is your employment status?

- Full time
- Part time
- Self employed
- Retired
- Student
- Unemployed
- Other (write in): \_\_\_\_\_

Q22: What is your highest level of education completed?

- Some high school
- High school graduate
- Some college
- Vocational certificate or 2-year degree
- 4-year degree
- Graduate degree

Q20: In which sector are you employed?

- Government
- Tribal
- Agriculture/Farming
- Fishing
- Education
- Retail
- Customer service
- Unemployed
- Retired
- Other (write in): \_\_\_\_\_

**THANK YOU!**

*You have reached the end of the survey.*



**QUESTIONS?**

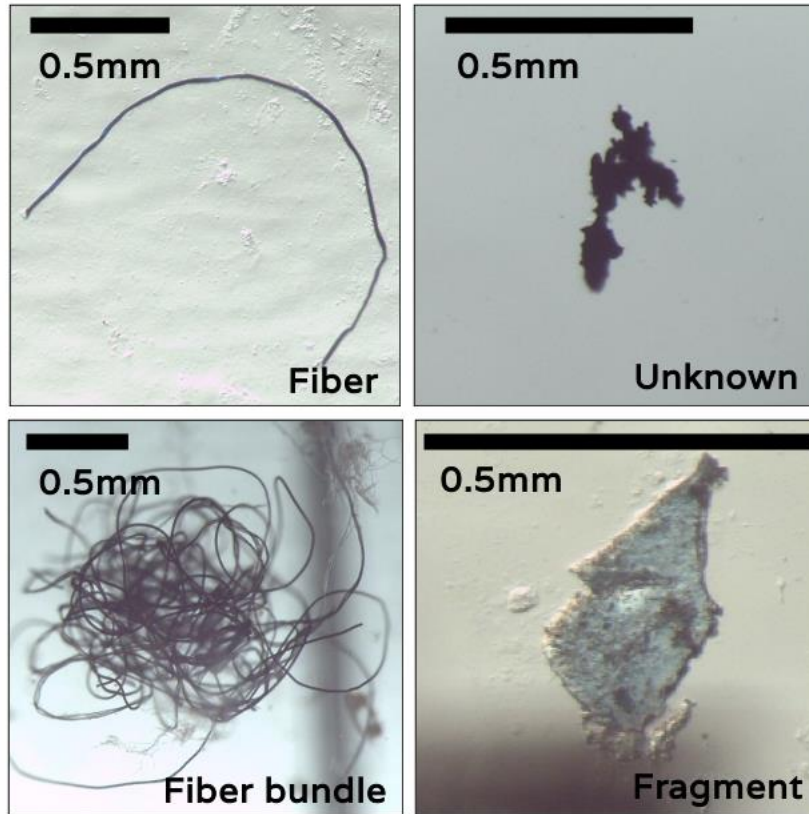
**Britta Baechler, PhD student  
Environmental Science & Mgmt. Program  
Portland State University  
baechler@pdx.ed**

**APPENDIX F: CHAPTER 3.** Number and percentage of Olympic Coast questionnaire respondents that consumed marine shellfish species or species groups. Consumption frequency is ordered from most to least frequent.

Consumption frequency	BIVALVES						CRUSTACEANS			GASTROPODS	ECHINODERMS
	Hardshell						Dungeness crab			Snails	Urchins
	Oysters	clams	Scallops	Mussels	Bay clams	Geoducks	crab	Shrimp	Other crab		
7+ meals/week	2	0	1	0	0	0	0	1	0	0	0
5 to 6 meals/week	0	0	0	0	0	0	1	0	0	0	0
2 to 4 meals/week	2	2	1	2	2	1	2	6	1	0	0
About 1 meal/week	1	1	0	0	0	0	2	6	2	0	0
2 or 3 meals/month	3	1	6	2	4	0	6	19	1	0	0
About 1 meal/month	9	7	8	5	4	0	14	24	7	0	0
< 6 meals/year	58	47	37	33	22	11	69	31	42	10	2
I don't know	1	2	0	0	2	0	0	0	0	0	0
Never	26	43	49	60	66	89	10	16	43	88	96
Total responses	102	103	102	102	100	101	104	103	96	98	98
Percentage of consumers <sup>1</sup>	73.5%	56.3%	52.0%	41.2%	32.0%	11.9%	90.4%	84.5%	55.2%	10.2%	2.0%

*Notes:* <sup>1</sup>Calculated as number of respondents that reported consuming a particular species or species group (i.e. all consumption frequencies except 'I don't know' and 'Never'), divided by total responses for that species or species group, multiplied by 100.

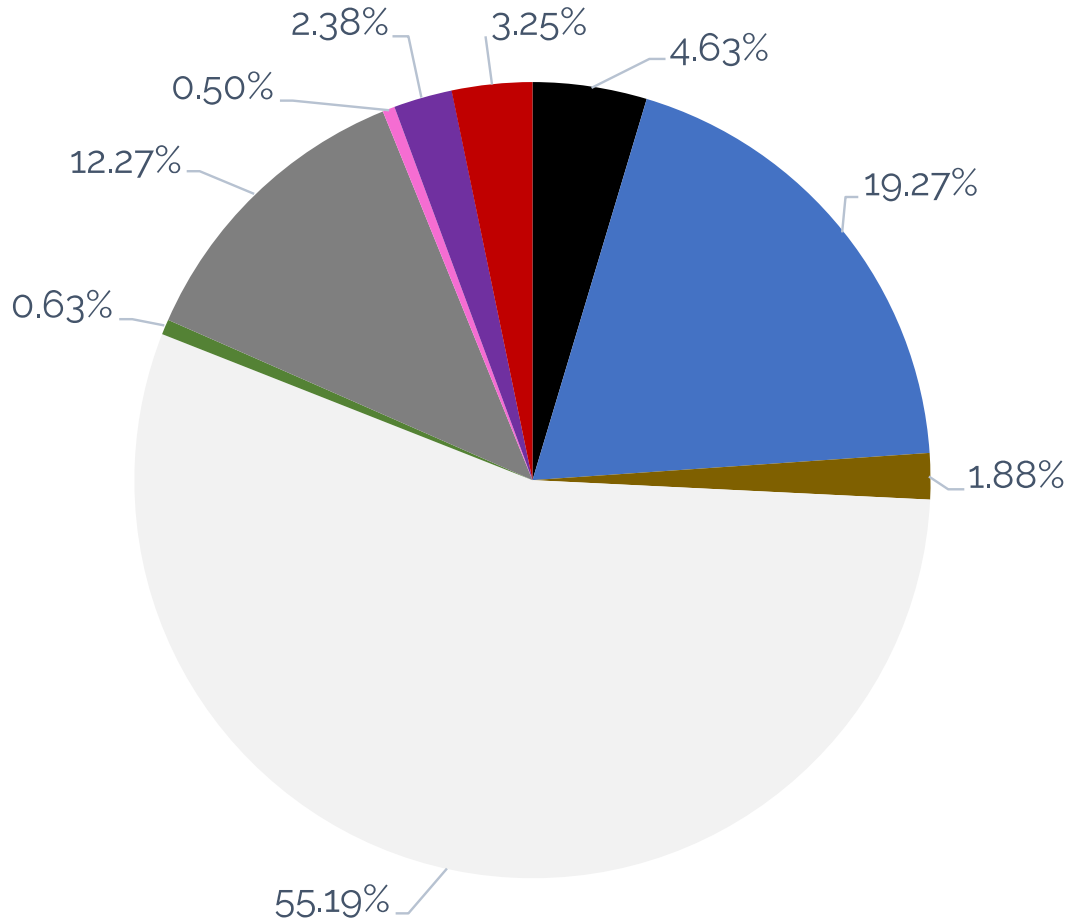
**APPENDIX G: CHAPTER 3.** Example photos of suspected microplastics (MPs) extracted from Olympic Coast razor clams (top) and total counts of each MP category encountered (bottom) in the 4 razor clam sample types included in this study (whole, cleaned, gut-tissue only, tissue-only). Black bars represent a length of 0.5 mm in each of the images shown.



<b>MP Category</b>	<b>Total #</b>
Fiber	793
Unknown	3
Fiber bundle	2
Fragment	1
<b>Total</b>	<b>799</b>



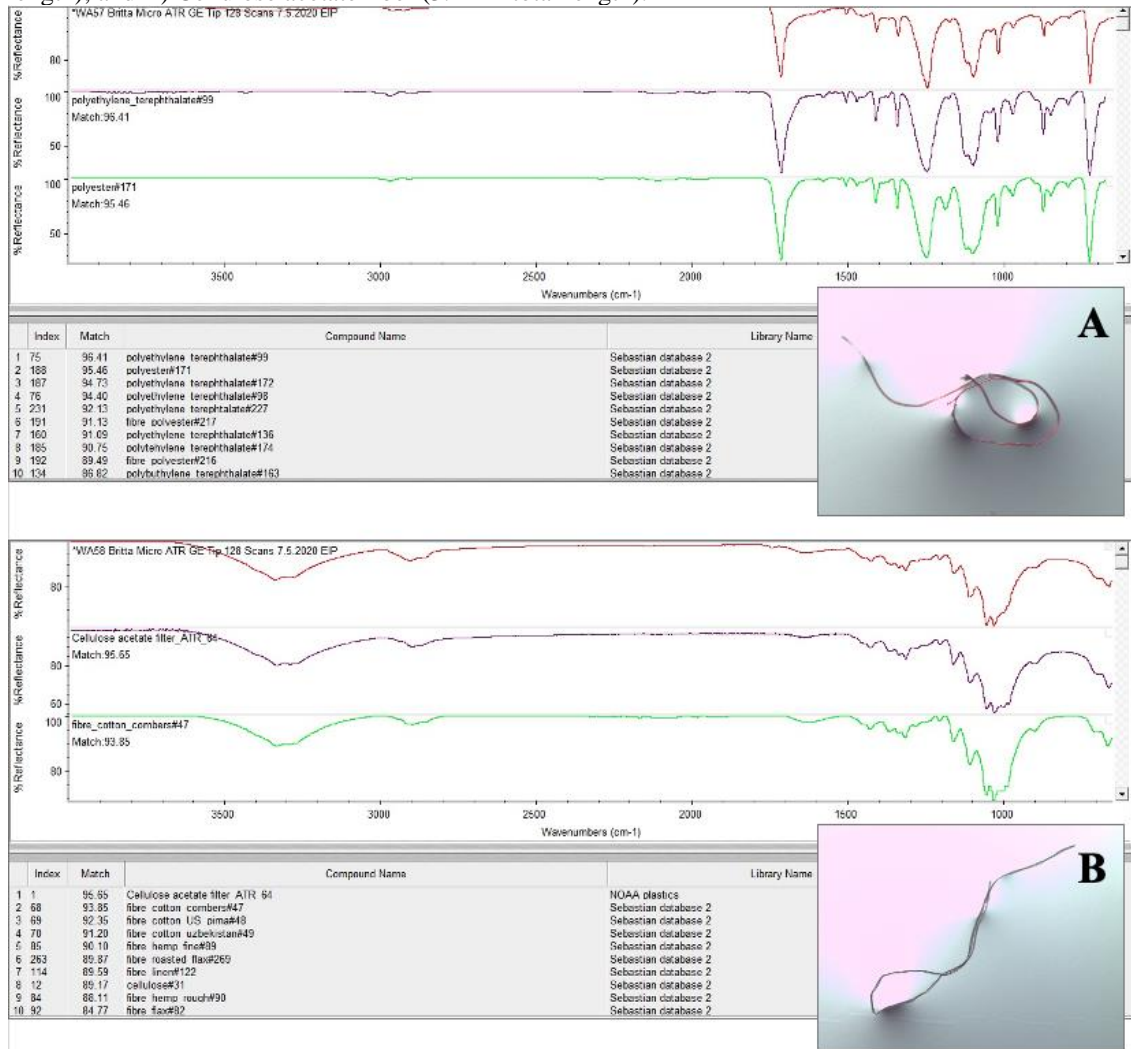
**APPENDIX H: CHAPTER 3.** Numbers and percentages of suspected microplastics identified in 138 Olympic Coast razor clam samples (n=799) by color. Colors in figure correspond to actual colors encountered: colorless (n= 441; 55.19%), blue (n=154; 19.27%), gray (n=98; 12.27%), black (n=37; 4.63%), red (n=26; 3.25%), purple (n=19; 2.38%), brown (n=15; 1.88%), green (n=5; 0.63%), and pink (n=4; 0.50%).



**APPENDIX I: CHAPTER 3.** Characteristics, material types and FTIR spectral match percentages for 53 randomly-selected suspected microplastics isolated from Olympic Coast razor clams. Results are sorted by FTIR % top match from highest to lowest.

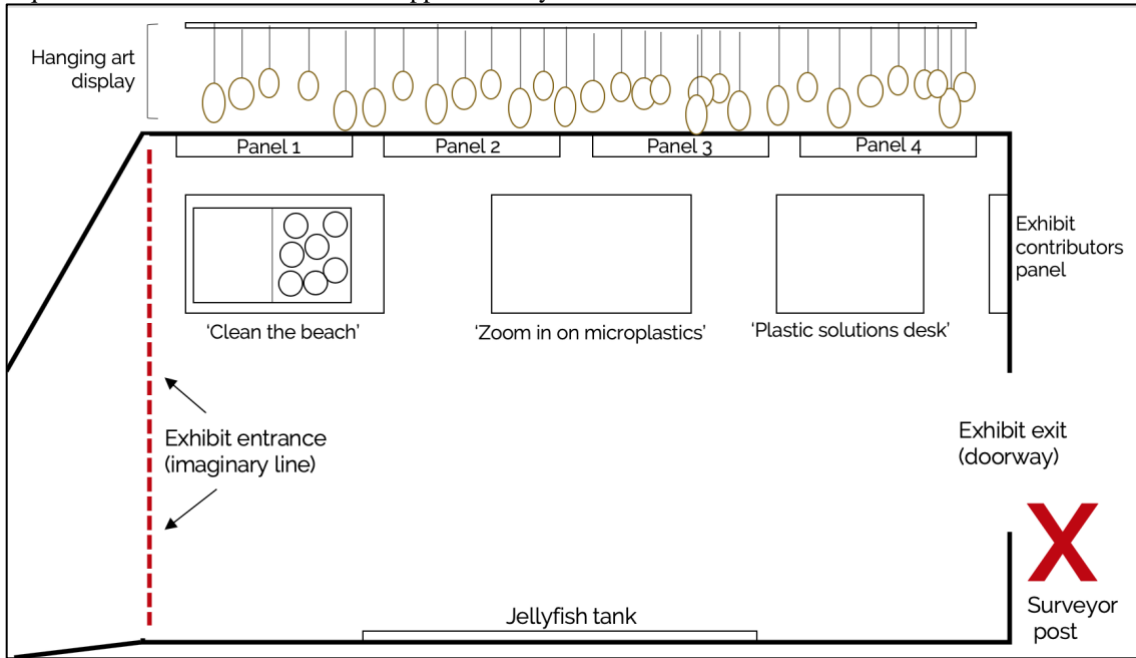
<b>Color</b>	<b>Length (mm)</b>	<b>Material type determined</b>	<b>FTIR % match</b>
Red	1.9	Polyethylene terephthalate	96.41%
Gray	3.2	Cellulose acetate	95.64%
Brown	1.3	Polyethylene terephthalate	95.49%
Colorless	1.2	Polyethylene terephthalate	95.37%
Red	2.8	Cellulose acetate	95.24%
Colorless	7.8	Polyester	95.19%
Black	0.9	Polyethylene terephthalate	94.93%
Gray	2.7	Cellulose acetate	94.66%
Colorless	1.8	Polyethylene terephthalate	94.60%
Colorless	0.8	Polyethylene terephthalate	94.02%
Colorless	1.1	Polyester	93.14%
Colorless	0.6	Polyethylene terephthalate	92.45%
Colorless	0.7	Polyethylene terephthalate	92.10%
Colorless	2.9	Cellulose	92.09%
Colorless	2.2	Cellulose acetate	91.95%
Black	0.6	Polyethylene terephthalate	91.85%
Colorless	1.5	Polyethylene terephthalate	91.32%
Colorless	0.7	Cellulose	90.61%
Red	6.4	Cellulose acetate	90.39%
Colorless	1.0	Cellulose acetate	89.96%
Black	0.8	Cellulose acetate	89.85%
Colorless	1.9	Polyethylene terephthalate	89.55%
Colorless	1.9	Polyethylene terephthalate	89.33%
Blue	0.4	Polyethylene terephthalate	89.21%
Colorless	1.1	Polyethylene terephthalate	88.35%
Colorless	1.4	Cellulose	87.98%
Colorless	0.7	Polyethylene terephthalate	87.82%
Blue	0.7	Polyethylene terephthalate	87.69%
Colorless	0.7	Polyethylene terephthalate	87.15%
Blue	1.4	Cellulose acetate	86.86%
Colorless	1.0	Polyethylene terephthalate	86.77%
Gray	1.7	Polyethylene terephthalate	86.73%
Colorless	1.2	Nylon	86.32%
Colorless	0.9	Polyethylene terephthalate	85.80%
Gray	2.6	Cellulose	85.65%
Colorless	1.1	Polyethylene terephthalate	85.64%
Blue	2.2	Cellophane	85.48%
Colorless	1.2	Cellulose	84.47%
Colorless	0.8	Cellulose	82.95%
Blue	1.5	Cellulose acetate	82.50%
Colorless	4.5	Polyester	81.98%
Gray	0.9	Cellophane	81.26%
Blue	2.5	Cellulose	80.00%
Blue	1.0	Cellulose	79.00%
Gray	1.6	Cellulose acetate	78.46%
Colorless	1.2	Cellulose	78.12%
Colorless	1.7	Cellulose acetate	77.02%
Blue	1.8	Cellophane	74.75%
Blue	2.3	Cellulose	74.39%
Gray	1.0	Cellophane	74.32%
Black	2.4	Cellophane	71.93%
Blue	1.1	Cellophane	71.90%
Gray	1.3	Low spectral match	64.29%

**APPENDIX J: CHAPTER 3.** Example FTIR-ATR spectra of microplastics isolated from Olympic Coast razor clams, stacked along with the two highest percentage matches from Omnic, Primpke et al. 2018 and Primpke et al. 2020 FTIR polymer databases for a: A) Polyethylene Terephthalate fiber (1.9mm total length); and B) Cellulose acetate fiber (3.2mm total length).



Chapter 4

**APPENDIX K: CHAPTER 4.** Aerial schematic of the ocean plastic exhibit at the Oregon Coast Aquarium. Exhibit dimensions were approximately 12 x 10 ft.



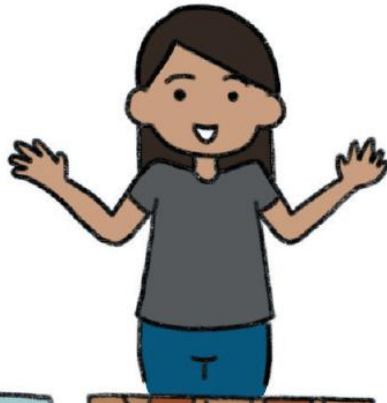
**APPENDIX L: CHAPTER 4.** Layout and content of wall panels at the Oregon Coast Aquarium ocean plastic exhibit.

**APPENDIX M: CHAPTER 4.** Cartoon zine booklet created by Nini Bui and Britta Baechler for the ocean plastic exhibit at the Oregon Coast Aquarium. A laminated, spiral-bound copy was mounted to the *Plastic Solutions Desk* with paracord.

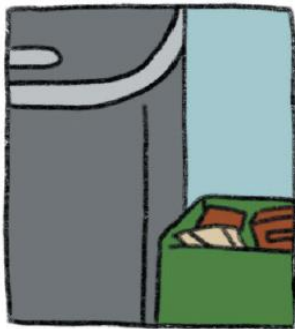




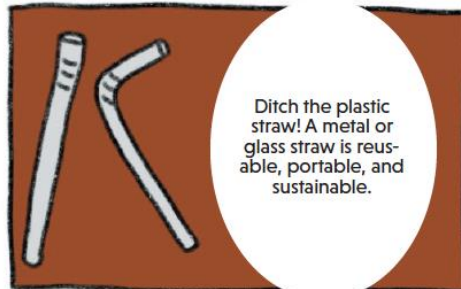
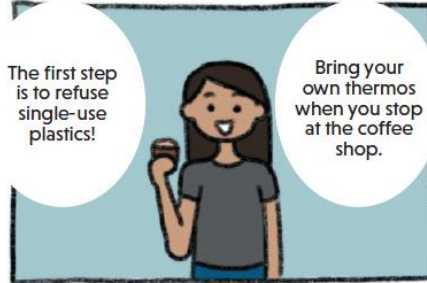
Hello!



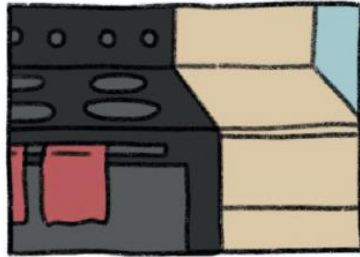
Today I will be teaching you about different things you can do to reduce your use of plastic.



# Refuse



# Reduce



We can't always control how our food is packaged when we buy it, but we can control how we store our food.



Food-safe canvas bags are reusable and great for storing snacks!



Paper coated in beeswax is a substitute for plastic wrap. Use it to wrap up bread and baked goods.



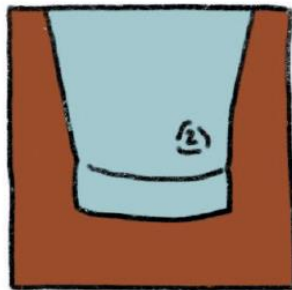
Glass mason jars are a great alternative to plastic. They are easy to clean and useful for storing everything from soups to cereal.



These are just a few earth-friendly examples to help you reduce your use of plastic at home.



# Recycle



# Restore



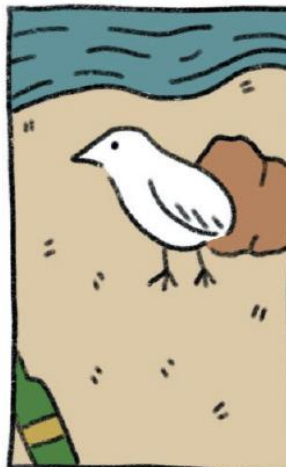
Unfortunately, trash is a common sight on the beach. You can help restore the beach to a clean place.



Join family, friends, or conservation groups at beach cleanups! You are instantly making a difference!

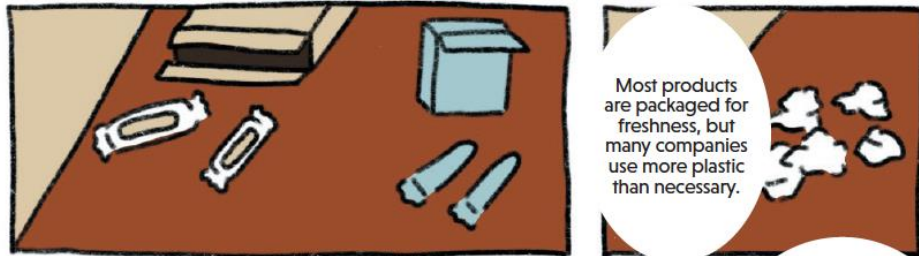


Picking up trash keeps it from breaking into small pieces that animals mistake for food.



When you pick up trash, the beach is cleaner and safer for wildlife!

# Represent





Nini Bui is a graphic design student at Portland State University. Her work focuses on storytelling in all forms. She loves to tell her own stories along with helping others share theirs.

Her portfolio can be found at [nibui.myportfolio.com](https://nibui.myportfolio.com).

## Other Resources

**Oregonians standing up to plastic waste:**  
<https://oregoniansagainstwaste.com/>

**Check out the Oregon Marine Debris Action Plan!**  
<https://marinedebris.noaa.gov/discover-issue>

**Play with this app to reduce your plastic footprint:**  
<https://mylittleplasticfootprint.org/>



**APPENDIX N: CHAPTER 4.** Structured questionnaire administered to Oregon Coast Aquarium visitors in August and November 2019.

SURVEY ID.: \_\_\_\_\_

Interview date: \_\_\_\_ / \_\_\_\_ /'19 Time: \_\_\_\_:\_\_\_\_ AM PM Interviewer initials: \_\_\_\_\_

1. Is this your first time to the Oregon Coast Aquarium? Yes No I don't know

2. Was this your first time seeing the Marine Plastics exhibit? Yes No I don't know

1A. IF NO, how many times have you visited the exhibit before? \_\_\_\_\_

3. What do you think the Aquarium was trying to get across to people with this exhibit?

5. How would you finish this sentence: *"Before visiting this exhibit, I never realized that..."*

6. How would you finish this one: *"Seeing this exhibit reminded me that..."*

7. What did **you** like best about the exhibit?

8. On a scale of 'Strongly disagree' to 'strongly agree': **I learned from interacting with this exhibit**

Strongly Disagree Disagree Neutral Agree Strongly Agree

9A. If children in the group interacted with the exhibit, what did **they** like best?

9B. (If children in the group interacted with exhibit– ask parents): Please answer this statement on a scale of 'Strongly disagree' to 'strongly agree': **My child learned from interacting with this exhibit**

Strongly Disagree Disagree Neutral Agree Strongly Agree

SURVEY I.D.: \_\_\_\_\_

9C. (Only if their rating on 9B is 'Disagree' or 'Strongly disagree': Can you describe why you think the exhibit didn't work well for your child?

10. Were there any parts of the exhibit that you felt were confusing, and how can the exhibit be improved?

15. How would you rate your overall impression of this exhibit? (0-4, 0 is low, 4 is high)

0      1      2      3      4

11A. Before coming to this exhibit, how would you have rated your desire to help address the marine plastics problem? (0-4, 0 is low, 4 is high)

0      1      2      3      4

11B. How would you rate your desire to help address the marine plastics problem now? (0-4, 0 is low, 4 is high)

0      1      2      3      4

11C. (Only if their rating on Q11B is lower than Q11A) I noticed you rated your interest lower after seeing the exhibit. Can you describe why you think that is?

13. What are the first two positive words that come to mind when you hear the word 'plastic'?

\_\_\_\_\_

14. What are the first two negative words that come to mind when you hear the word 'plastic'?

\_\_\_\_\_

**QUESTIONS 4 & 17-20 on separate sheet (MAKE SURE SURVEY ID is present)**



SURVEY I.D.: \_\_\_\_\_

**Question 4: How often do you do the following things in your life?  
(Circle one number per horizontal row)**

	N/A	Never	Rarely, I have done this a couple of times	Sometimes, about once or twice a week	Frequently, three or more days a week	Almost everyday
Carry a reusable water bottle		0	1	2	3	4
Bring your own bag to the store or market		0	1	2	3	4
Use a reusable straw (ex: metal, glass, bamboo)		0	1	2	3	4
Bring your own refillable coffee container		0	1	2	3	4
Choose items not packaged in plastic when available		0	1	2	3	4
Specifically ask for your drink to come without a straw		0	1	2	3	4
Bring your own containers to restaurants for take-out food		0	1	2	3	4
Carry your own utensils (forks, spoons, knives)		0	1	2	3	4
Clean the beach (informal or formal beach cleanups)		0	1	2	3	4
Contact companies or representatives to encourage plastic reduction		0	1	2	3	4

After completing the table, place 1 or 2 stars to the left of the actions you expect to start doing or increase (**1 star= probably will do, 2 stars= definitely will do**).

SURVEY I.D.: \_\_\_\_\_

18. Who are you visiting the aquarium with today? (Check 1 box)

- Family or friends (including kids younger than 18)
  - If kids in the group, what are their ages? (please write in) \_\_\_\_\_
- Family or friends (all adults)
- By myself
- Organized group (camp, scouts, tour, etc.)

19. What is your gender? (Circle one)    Female            Male            I identify my gender differently

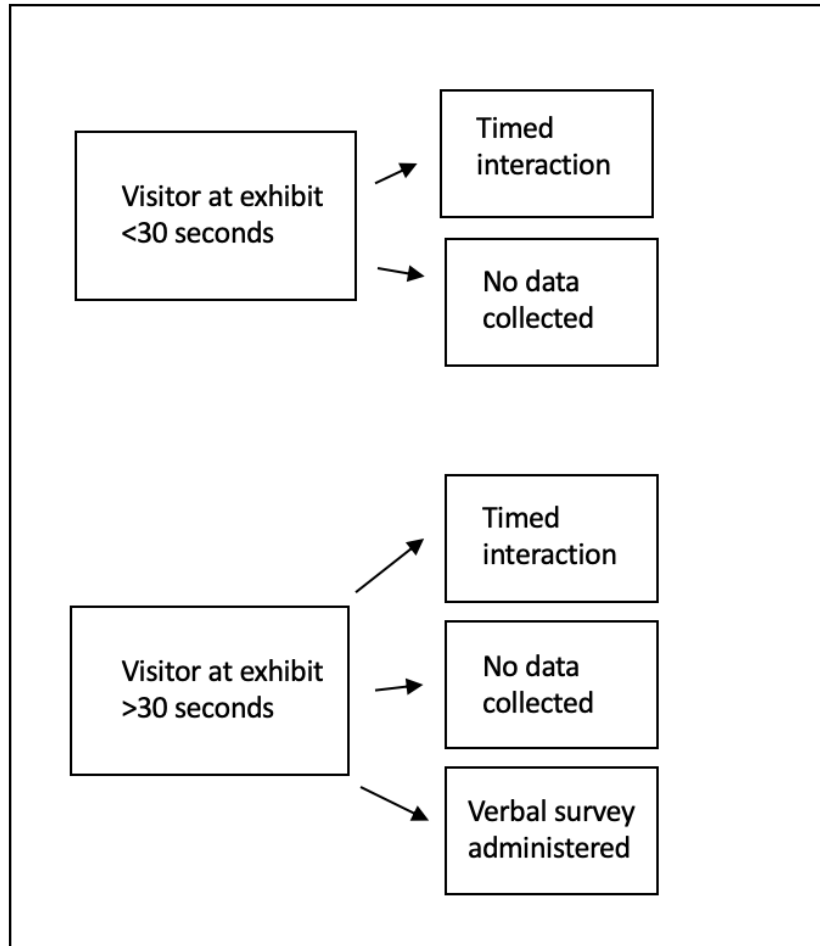
20. What is your age? (Circle one)    18-29    30-39    40-49    50-59    60-69    70-79    80+

21. What is your zipcode? \_\_\_\_\_

\*\*\*\*\* Survey complete. Thank you for participating! \*\*\*\*\*



**APPENDIX O: CHAPTER 4.** Conceptual diagram showing data collection options for Oregon Coast Aquarium visitors entering the ocean plastic exhibit. Structured questionnaires were administered to randomly selected individuals that verbally confirmed they were 18 years of age or older.



**APPENDIX P: CHAPTER 4.** Datasheet for timed interaction survey data collected at the Oregon Coast Aquarium in August and November 2019.

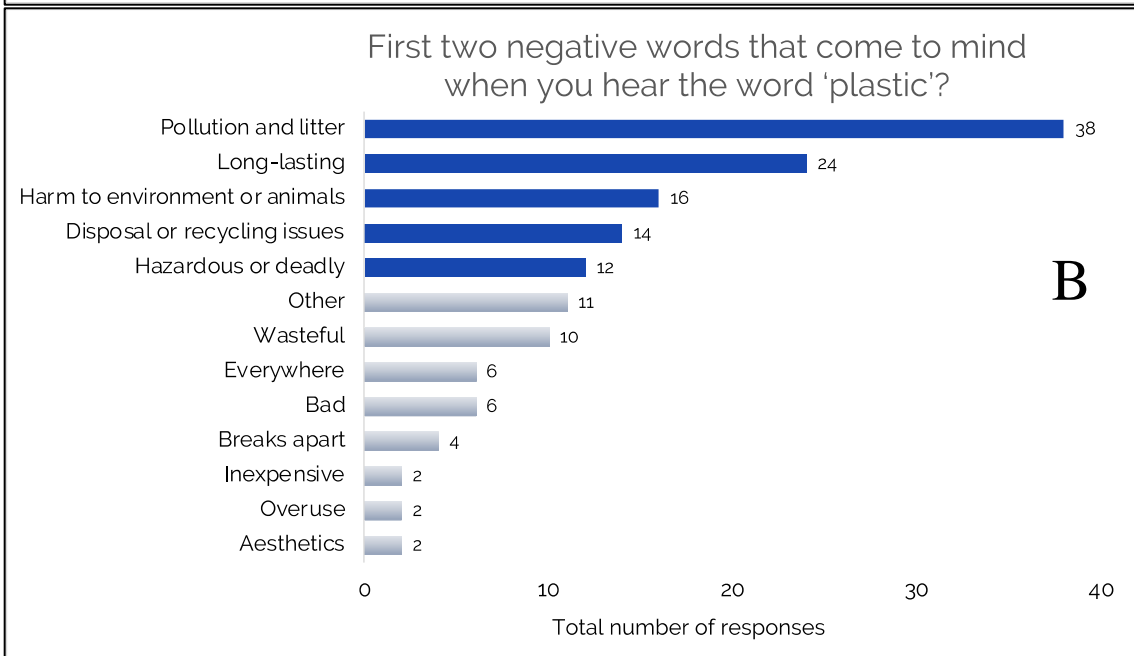
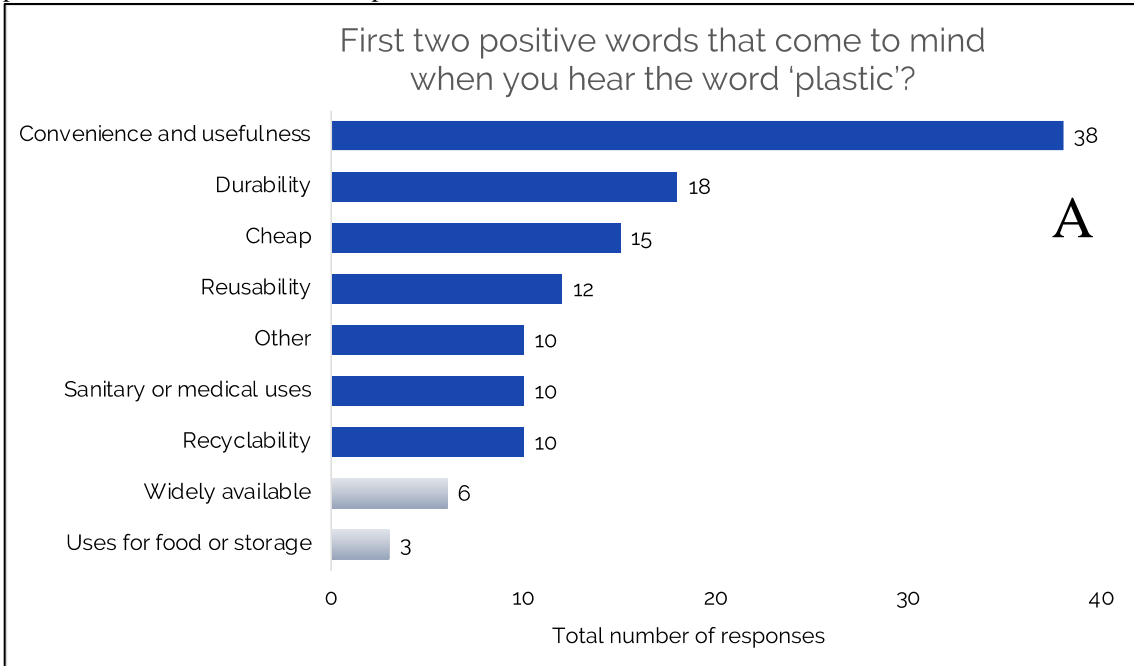
Timed interaction survey									
Surveyor initials: _____									
Date: ____/____/2019									
Page # for day: _____									
Survey start time (12-hr clock)	Crowding level (low, med or high)	Total time in exhibition before turning away (mins & seconds)	#times touched sand table (tally)	#times touched scope table (tally)	#times touched solutions desk (tally)	#times looked at wall panels (tally)	Which panels looked at (circle all)	Age range (0-5 6-10 11-17 18-29 30s 40s 50s 60s 70s 80+)	Gender (M, F, ?)
1							1 2 3 4		
2							1 2 3 4		
3							1 2 3 4		
4							1 2 3 4		
5							1 2 3 4		
6							1 2 3 4		
7							1 2 3 4		
8							1 2 3 4		
9							1 2 3 4		
10							1 2 3 4		
NOTES:									

**APPENDIX Q: CHAPTER 4.** Categorizations for open-ended Oregon Coast Aquarium ocean plastic exhibit questionnaire responses (Questions 3, 5, 6, 7, 9A, 10, 13, 14) thematically coded by the Applied Coastal Ecology (ACE) lab group at Portland State University. A percent agreement score for each question was generated, calculated as the percent similarity of response categorization between ACE and the paper's lead author. Final categorizations were determined by the lead author.

<b>Question #</b>	<b>Question posed</b>	<b>Number of responses</b>	<b>Number of categorizations</b>	<b>Number of categories</b>	<b>Percent agreement</b>
3	"What do you think the Aquarium was trying to get across to people with this exhibit?"	92	156	16	<b>94.87%</b>
5	"Before visiting this exhibit, I never realized that..."	84	116	13	<b>84.48%</b>
6	"Seeing this exhibit reminded me that..."	87	136	13	<b>85.29%</b>
7	"What did you like best about the exhibit?"	93	115	9	<b>87.83%</b>
9A	"If children in the group interacted with the exhibit, what did they like best?"	66	100	6	<b>94.00%</b>
10	"Were there any parts of the exhibit that you felt were confusing, and how can the exhibit be improved?"	93	109	9	<b>90.83%</b>
13	"What are the first two positive words that come to mind when you hear the word 'plastic'?"	144*	144	10	<b>97.22%</b>
14	"What are the first two negative words that come to mind when you hear the word 'plastic'?"	164*	164	14	<b>95.12%</b>

*Note:* \*= Represents up to two answers per respondent.

**APPENDIX R: CHAPTER 4.** Thematically categorized open-ended responses by 93 Oregon Coast Aquarium visitors for the first two- A) positive words that come to mind when you hear the word 'plastic'; and B) negative words that come to mind when you hear the word 'plastic'. Top five responses by frequency are represented in blue, remaining responses are represented in gray. Not all respondents provided two words each. Non-responses were not included.



**APPENDIX S: CHAPTER 4.** Mean, Median and Mode responses for 91 Oregon Coast Aquarium visitors who self-reported single-use plastic reduction or plastic stewardship actions. Actions are sorted left to right from highest to lowest mean response. 0= Never; 1= Rarely (I have done this a couple of times); 2= Sometimes (about once or twice a week); 3= Frequently (three or more days a week); 4= Almost every day.

	Carry a reusable water bottle	BYO coffee container	BYO bag to store or market	Choose items not packaged in plastic	Specifically ask for no straw	Use a reusable straw	Clean the beach	Carry your own utensils	Contact companies or representatives	BYO containers to restaurants
<b>Mean</b>	3.4	2.6	2.5	2.1	1.7	1.6	1.5	1.0	0.4	0.4
<b>Median</b>	4	3	3	2	1	1	1	0	0	0
<b>Mode</b>	4	4	4	2	0	0	1	0	0	0

*Note:* BYO= Bring your own. NAs and non-responses were not included in mean, median or mode response calculations.