LIMNOLOGY AND OCEANOGRAPHY
Letters

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Limnology and Oceanography Letters 2019
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doi: 10.1002/lol2.10124

SPECIAL ISSUE-LETTER

Microplastic concentrations in two Oregon bivalve species: Spatial, temporal, and species variability

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Scientific Significance Statement

Plastics have innumerable uses and are inextricably tied to daily life in modern society. These plastics begin as or break down into microplastics, which are now found in an array of terrestrial, aquatic, and marine habitats and organisms. These tiny particles may threaten ecosystem balance and natural resource consumers, particularly in the case of seafood. In Oregon, U.S.A., Pacific oysters (*Crassostrea gigas*) and razor clams (*Siliqua patula*) are of commercial, recreational, and cultural importance, yet baseline information on microplastic prevalence in these species and across sites and seasons is absent. Our study is the first to document microplastics in Pacific razor clams and provides important coast-wide data to compare microplastic burden across species, seasons, and sites.

Abstract

Microplastics are an ecological stressor with implications for ecosystem and human health when present in seafood. We quantified microplastic types, concentrations, anatomical burdens, geographic distribution, and temporal differences in Pacific oysters (*Crassostrea gigas*) and Pacific razor clams (*Siliqua patula*) from 15 Oregon coast, U.S.A. sites. Microplastics were present in organisms from all sites. On average, whole oysters and razor clams contained 10.95 ± 0.77 and 8.84 ± 0.45 microplastic pieces per individual, or 0.35 ± 0.04 pieces g^{-1} tissue and 0.16 ± 0.02 pieces g^{-1} tissue, respectively. Contamination was quantified but not subtracted. Over 99% of microplastics were fibers. Material type was determined using Fourier-transform infrared spectroscopy. Spring samples contained more microplastics than summer samples in oysters but not razor clams. Our study is the first to document microplastics in Pacific razor clams and provides important coast-wide data to compare microplastic burden across species, seasons, and sites.

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Author Contribution Statement: B.R.B. and E.F.G. identified the research question and study approach. M.V.H., E.F.G., and B.R.B. designed the field survey; M.V.H. and B.R.B. conducted the field survey. K.E.C., E.F.G., and B.R.B. designed laboratory methods; B.R.B. conducted the laboratory study. B.R.B. led the manuscript effort; E.F.G., M.V.H., and K.E.C. contributed ideas and edits to the manuscript. B.R.B. and E.F.G. are responsible for manuscript data.

Data Availability Statement: Data are available in the PDXScholar data repository at https://doi.org/10.15760/esm-data.1.

Associate editor: Erika Holland

Additional Supporting Information may be found in the online version of this article.

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This article is an invited paper to the Special Issue: Microplastics in marine and freshwater organisms: Presence and potential effects
Edited by: Dr Elise Granek, Portland State University, Dr Susanne Brander, Oregon State University, and Dr Erika Holland, California State University, Long Beach

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 nondegradable 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 distribution of microplastics in the environment is 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 (Jambeck et al. 2015; Zhang 2017). Density has been thought to ultimately determine environmental fate, with denser plastics like polyvinyl chloride and polyethylene terephthalate (PET) settling to the benthos and low density polymers such as polystyrene, polypropylene, and polyethylene remaining in surface waters; however, a recent review of global surface water and sediment data indicates 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 (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 2 billion microplastic pieces into coastal waters in the span of just 3 d (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., Carr et al. 2016; Napper and Thompson 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 1900s (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-yr lifespan (Link 2000).

Microplastic concentrations in field-collected Pacific oysters have been documented worldwide facilitating comparisons between samples grown in Oregon vs. 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.A. 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–October). Tourism results in 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 nongut 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 than smaller individuals. 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.

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 (27–28 April 2017) and summer (21–31 July 2017) (Table 1). Whole oysters were purchased from growers at six 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; Supporting Information Appendix 1).

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 (Fig. 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 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; Supporting Information Appendix 1).

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 deionized (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 (330 g L^{-1} Fisher Chemical Certified ACS Crystalline NaCl) was utilized. Samples were analyzed under a Leica M165C stereomicroscope (×10–120 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 category (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 256–1024 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

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.

		Spring					Summer					Both seasons		
		# samples analyzed			Microplastic burden		# samples analyzed			Microplastic burden				
Species	Site	Whole organisms	Gut tissue	Nongut tissue	Avg # MP per sample (SE)[1]	Avg # MP g^{-1} tissue (SE)[2]	Whole organisms	Gut tissue	Nongut tissue	Avg # MP per sample (SE)[1]	Avg # MP g^{-1} tissue (SE)[2]	Avg MP length in mm (SE)[3]	Min MP length (mm)[4]	Max MP length (mm)[5]
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; OY1–OY6: oyster site (randomized). Reported values include background and processing fiber levels.
[1]Average number of MP per sample (SE).
[2]Average number of MP per gram of tissue (SE).
[3]Average MP length in millimeters (SE).
[4]Minimum MP length at site in millimeters (SE).
[5]Minimum MP length at site in millimeters (SE).

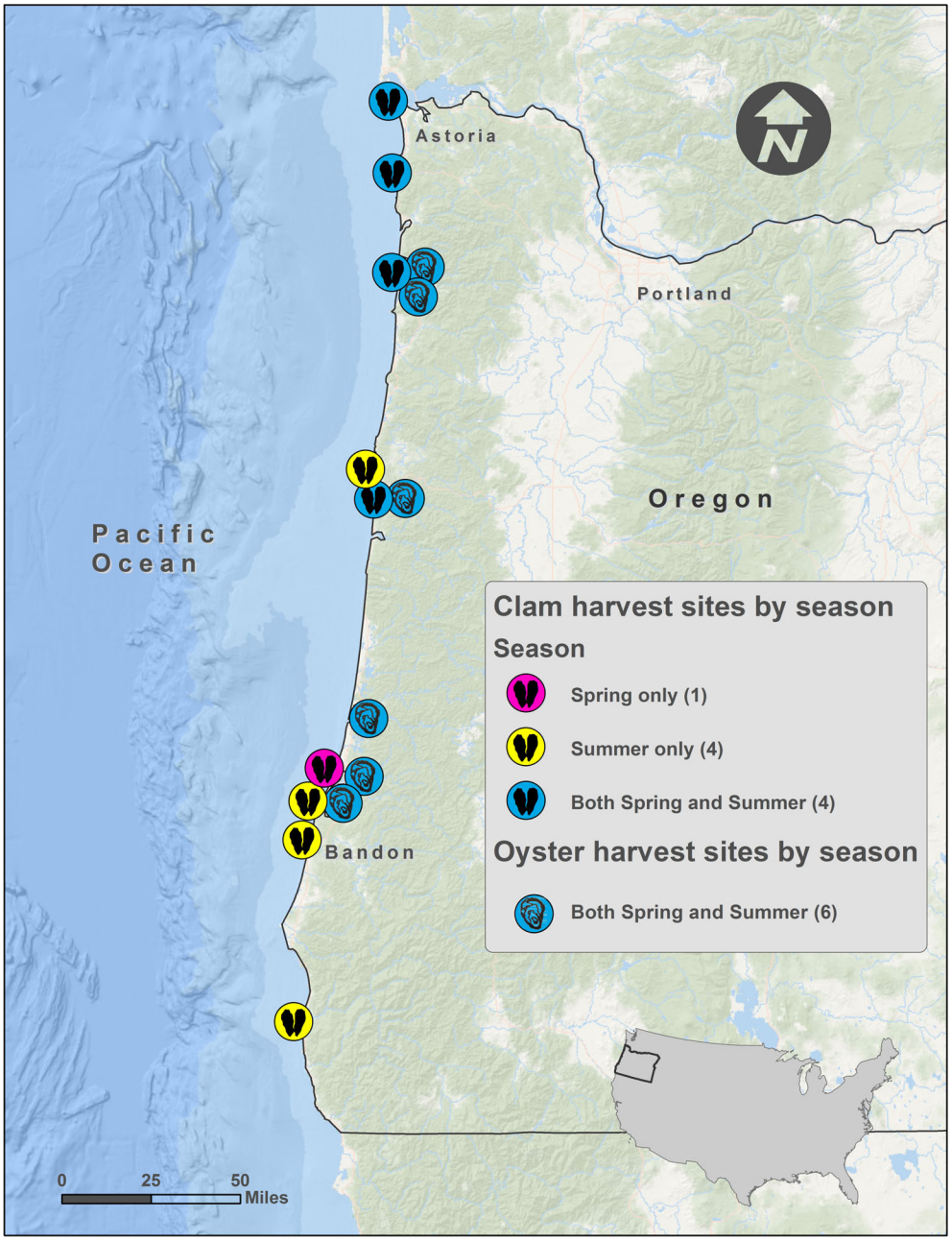

Fig. 1. The 2017 sample collection sites along the Oregon coast delineated for Pacific oysters and Pacific razor clams (Map credit: K. Scully-Engelmeyer; Service Layer Credits: Esri, Garmin, GEBCO, NOAA, NGDC, and other contributors; Sources: Esri, USGS, NOAA).

classified as nongut tissue. Separated gut and nongut 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 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.

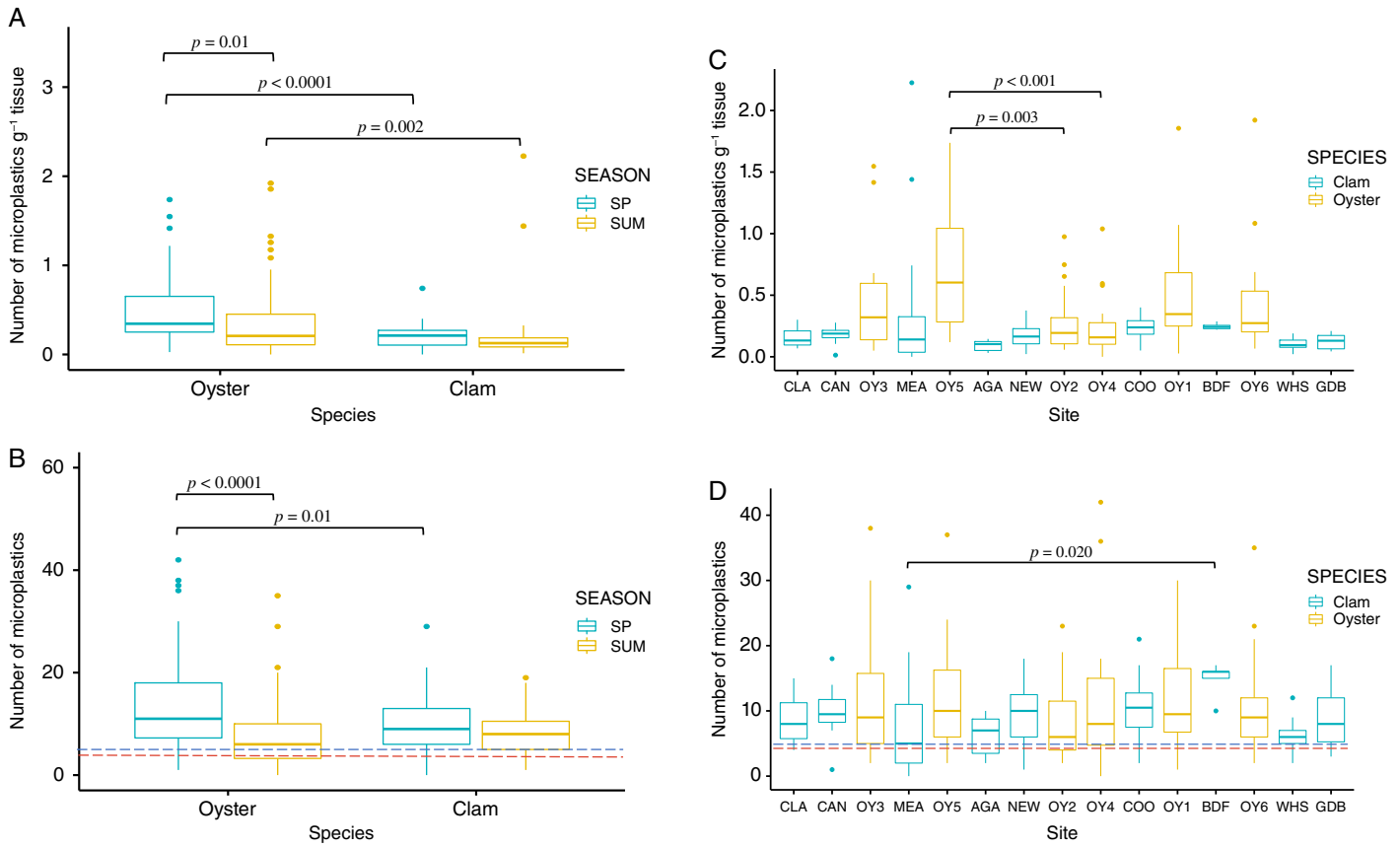

Fig. 2. (**A**, **B**) Number of microplastics by season (SP = spring [teal] and SUM = summer [gold]) and species for Oregon Pacific oysters and Pacific razor clams: (**A**) per gram of whole-organism tissue, and (**B**) per whole organism. Welch's *t*-tests were run to determine seasonal intraspecies and interspecies 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.

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, 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 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.

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 ± 1.11), microscope blanks (0.20 ± 0.03), and, for razor clams, a secondary digestion and separation step

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	df	Significance	Test type	Test statistic	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	**$p = <0.0001$**	*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	**$p = <0.0001$**	*t*-test	$t = -2.63$	df = 103	**$p = <0.001$**
	Whole oyster to whole razor clam (summer only)	*t*-test	$t = -3.24$	df = 103	**$p = 0.002$**	*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	**$p = 0.01$**	*t*-test	$t = 4.41$	df = 121	**$p = <0.0001$**
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.

(1.0 ± 0.0) was quantified (Supporting Information Appendix 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 ± 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, 2016, 2018*a*; Davidson and Dudas 2016; Qu et al. 2018; Su et al. 2018; Rochman et al. 2019), we report microplastics detected in blank samples (Supporting Information Appendix 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 nongut tissue samples. Over 99% of particles were microfibers ($n = 3,026$) averaging 1.34 mm 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, Fig. 2).

Mean microplastic concentrations in whole organisms was 10.95 ± 0.77 in Pacific oysters (range = 0–42) and 8.84 ± 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 ± 0.04 g^{-1} tissue) and razor clams (0.16 ± 0.02 g^{-1} tissue; $t = -6.43$, df = 199; $p \leq 0.0001$), 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 PET ($n = 8$), 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.

Temporal differences

Significant intraspecies and interspecies differences in microplastic burdens were detected during the two sampling periods (Fig. 2A,B). Spring Pacific oysters contained significantly more microplastics than summer; on average, whole spring oysters contained 13.74 ± 1.16 microplastics (0.45 ± 0.05 g^{-1} tissue) whereas summer oysters contained

8.16 ± 0.88 (0.26 ± 0.05 g^{-1} tissue; whole organism: $t = 4.41$; df = 121; $p < 0.0001$; MP 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 ± 0.81 microplastics per whole individual (0.19 ± 0.02 g^{-1} tissue) whereas summer had 8.35 ± 0.51 (0.14 ± 0.04 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; $p \leq 0.0001$) 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; $p = 0.19$).

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 (Fig. 2C; $p < 0.001$ and $p = 0.003$). Site-specific differences in number of microplastics per individual were not detected in oysters (Fig. 2D; $F = 0.56$; df = 5; $p = 0.73$). For razor clams, site-specific differences in microplastics per gram of tissue were not detected (Fig. 2C), but were for microplastics per individual in one site pairing (Fig. 2D; $F = 2.54$; df = 8; $p = 0.020$).

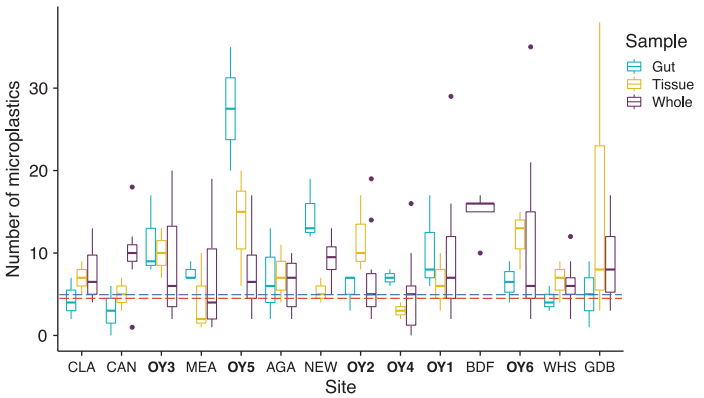

Fig. 3. Comparison of sample types for summer-collected Pacific oysters (site names bolded) and Pacific razor clams. Reported values include background and processing fiber levels. Gut = gut-tissue; Tissue = nongut tissue; Whole = whole organism. Dashed blue line indicates average contamination level 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.

Anatomical burdens

Microplastics were detected in whole organism, gut-tissue, and nongut 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 (Fig. 3). Microplastic burden (number of plastics per sample) did not differ between gut-tissue and nongut 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 ± 2.01 in gut-tissue and 9.41 ± 1.30 in nongut tissue samples. In razor clams, average microplastic burden was 6.57 ± 1.02 in gut-tissue and 7.43 ± 1.64 in nongut tissue samples.

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, $R^2 = 0.003$, $p = 0.247$).

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. 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 nongut tissues in either species, and organism size did not correlate with microplastic burden. Both Pacific oysters and razor clams 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 oysters and razor clams 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; Van Cauwenberghe and Janssen 2014; Li et al. 2015; Davidson and Dudas 2016; Su et al. 2018). In this study, the average number of microplastics found in Pacific oysters and razor clams (0.35 ± 0.04 g^{-1} tissue and 0.16 ± 0.02 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 g^{-1} tissue (Van Cauwenberghe and Janssen 2014; Abidli et al. 2019;

Teng et al. 2019), mussels from China of 2.2–-2.4 items g^{-1} tissue (Li et al. 2015, 2016), and manila clams from British Columbia, Canada of 0.9–1.7 items g^{-1} 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^{-1} tissue (De Witte et al. 2014; Phuong et al. 2018), and are the low end of concentrations found in manila clams in China of 0.3–4.9 items g^{-1} tissue (Su et al. 2018). These patterns may 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 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 PNW 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 (29–40 mm 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 (50–55 mm length) during a depuration period of 2 h (Rist et al. 2018). While, in these examples, depuration was studied in bivalves smaller than our study organisms (Supporting Information Appendix 1), 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 2428 microfibers found in whole samples, which were subsequently identified as PET (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. Polyethyelene terephthalate, acrylic, and aramid fibers have been previously found in organisms (e.g., Li et al. 2015, 2018*a*; Nelms et al. 2018), and zein (a corn-based protein used in bioplastics) has been isolated from 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, 2018*a*; Ding 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 and 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 (1×10^{-6} to 1×10^{-4} mm), as particles between 1×10^{-4} and 2×10^{-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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Acknowledgments

We thank E. Foster for procedural and analytical guidance; A. Marquardt, M. Rogers, S. Rumrill of ODFW and N. Jensen of ODA for assistance with razor clam sample collection and coordination; P. Wilkinson at the University of New Hampshire for her FTIR work; K. Scully-Engelmeyer for mapping assistance; J. Dorsheimer of Thermo Scientific for spectral matching assistance; A. Strecker for use of her microscope; A. Bolm, S. Holland, C. Orr, J. Adelsheim, M. Rollins, C. Homer, M. Jauregui, H. Smiley, D. Van Leuven, and C. Tenorio for assistance in the lab and field; and the Applied Coastal Ecology lab for valuable input and support. We also thank the two anonymous peer reviewers and editorial team for providing comments that greatly improved the manuscript. Funding for this project was provided by Oregon Sea Grant (Award NA14OAR4170064), the Edward D. & Olive C. Bushby Scholarship, the OR American Fisheries Society, and the Portland State University Institute for Sustainable Solutions.

Submitted 20 March 2019
Revised 04 September 2019
Accepted 22 September 2019