



# Microplastics in bivalves and their habitat in relation to shellfish aquaculture proximity in coastal British Columbia, Canada

Garth A. Covernton<sup>1,\*</sup>, Brenna Collicutt<sup>2,3</sup>, Helen J. Gurney-Smith<sup>1,2,3,4</sup>,  
Christopher M. Pearce<sup>1,2,5</sup>, John F. Dower<sup>1</sup>, Peter S. Ross<sup>6</sup>, Sarah E. Dudas<sup>1,2,3,5</sup>

<sup>1</sup>University of Victoria, Victoria, BC V8P 5C2, Canada

<sup>2</sup>Vancouver Island University, Nanaimo, BC V9R 5S5, Canada

<sup>3</sup>Hakai Institute, Quadra Island, BC V0P 1H0, Canada

<sup>4</sup>St. Andrews Biological Station, Fisheries and Oceans Canada, St. Andrews, NB E5B 0E4, Canada

<sup>5</sup>Pacific Biological Station, Fisheries and Oceans Canada, Nanaimo, BC V9T 6N7, Canada

<sup>6</sup>Coastal Ocean Research Institute, Ocean Wise Conservation Association, Vancouver, BC V6B 3X8, Canada

**ABSTRACT:** Shellfish aquaculture often uses large amounts of plastic equipment and has been suggested as a potential source of microplastic contamination in the marine environment. To determine the influence of shellfish aquaculture on microplastic concentrations in bivalves and their environment, we compared microplastic particle (MP) concentrations in Manila clams *Venerupis philippinarum* and Pacific oysters *Crassostrea gigas* grown on commercial shellfish beaches with those in individuals of the same species grown on nearby non-aquaculture beaches in 6 regions of coastal British Columbia, Canada. MP concentrations did not differ between shellfish aquaculture and non-aquaculture sites for either bivalve species, sediment, or water samples. Plastic presence differed by site and oysters on sites with many synthetic anti-predator nets contained significantly, yet marginally, more MPs than those on sites without (0.05 vs. 0.03 g<sup>-1</sup> dry-tissue weight on average). However, analysis of suspected MPs using Fourier-transform infrared spectroscopy indicated a predominance of fibres from textiles (including nylon and polyester), which are not typically used in shellfish aquaculture, suggesting that this may be caused by the larger average body weight of oysters grown at non-aquaculture sites rather than by the degradation of aquaculture infrastructure.

**KEY WORDS:** Microfibre · Ingestion · Food safety · Mariculture

## 1. INTRODUCTION

Microplastics are a ubiquitous ocean contaminant and suggested to represent a potential threat to marine ecosystems and to human food safety and security (Barboza et al. 2018). They have been documented to occur in coastal British Columbia (BC), Canada, both in seawater, and in the bodies of zooplankton (Desforges et al. 2014, 2015). Sufficient evidence has demonstrated negative effects on animals following the ingestion of microplastics to warrant consideration of the environmental risks of con-

tinued microplastic pollution in the oceans (Chae & An 2017, Peng et al. 2017). Once ingested, microplastic particles (MPs) can affect a range of biological processes including feeding capacity, body condition, and reproductive output (Cole et al. 2015, Sussarellu et al. 2016, Welden & Cowie 2016). According to recent studies, although the majority of marine animals studied have been shown to ingest MPs to some degree, many may also egest them without showing any indication of harm (Imhof & Laforsch 2016, Bruck & Ford 2018, Santana et al. 2018).

\*Corresponding author: gcov@uvic.ca

Microplastics have been documented in a wide range of animals commonly consumed by humans including fish, shellfish, and chicken (Rochman et al. 2015, Huerta Lwanga et al. 2017). They also occur in other food items such as beer, honey, sugar, and salt (Liebezeit & Liebezeit 2013, 2014, Karami et al. 2017) as well as in the air (Dris et al. 2016). Although the effects of MP consumption on human health are unknown, some authors have suggested potential detrimental impacts of ingestion and inhalation (Wright & Kelly 2017, Prata 2018). Once ingested or inhaled, MPs may accumulate in the gut or lungs. If small enough, they may also translocate into other organs and tissues, triggering a local immune response and/or potentially causing chemical toxicity via the leaching of monomers, additives, and/or absorbed contaminants (Wright & Kelly 2017).

Fisheries and aquaculture industries represent potential sources of marine plastic pollution and pathways for MP contamination of seafoods, due to the extensive use of equipment made of synthetic materials (Lusher et al. 2017). In particular, shellfish aquaculture requires the use of ropes, rafts, floats, and trays that—due to low cost and durability—are usually composed of polystyrene, polypropylene, PVC, or high-density polyethylene (GESAMP 2016, Schoof & DeNike 2017). In some cases, cultured shellfish have been shown to contain higher MP concentrations than their wild counterparts (Mathalon & Hill 2014, Bendell 2015, Phuong et al. 2018), and it has been suggested that by using plastic infrastructure, shellfish aquaculture could be contaminating its own stock as well as the surrounding environment (Mathalon & Hill 2014, Castro et al. 2016). However, little is known about the extent to which shellfish aquaculture equipment contaminates the environment compared with other well-established sources, such as sewage effluent, urban runoff, and aerial dispersal (Dris et al. 2016, GESAMP 2016, Schoof & DeNike 2017, Gies et al. 2018).

The finding that MPs occur in shellfish has generated media attention and public concern about the potential health risks to humans of ingesting MPs via shellfish consumption (Lusher et al. 2017). Determining the source of such MPs and attempting to reduce the contamination of shellfish, along with a further appreciation of their role as sources of MPs relative to other foods, will be important steps towards thoroughly understanding their safety and security as a food source, as well as promoting trust among consumers that shellfish are safe to eat. The impact of shellfish aquaculture on local MP accumulation in shellfish may be driven by a wide range of factors

including geographic location, local oceanographic conditions, species, season, culture practices, and type/concentration of MPs. Accordingly, research is required to investigate these factors/considerations to determine aquaculture impacts on MP accumulation in shellfish.

The objectives of this study were to compare MP concentrations in shellfish, water, and sediment from shellfish aquaculture sites with nearby non-aquaculture sites in order to determine the relationship with environmental variables (i.e. sediment composition, amount of plastic at a site) and to investigate the source of MPs found in shellfish and their environment. We conducted a large-scale out-plant experiment, where shellfish (oysters and clams) individuals were collected from 1 site and transferred to aquaculture and non-aquaculture sites in 6 regions important for shellfish aquaculture in BC.

## 2. MATERIALS AND METHODS

### 2.1. Study area

The study was conducted in 6 regions in BC where shellfish aquaculture occurs: Discovery Islands, Okeover Inlet, Baynes Sound, Nanoose Bay, Ladysmith, and Clayoquot Sound (Fig. 1). Eleven shellfish aquaculture sites and 10 non-aquaculture sites, with varying degrees of macroplastics present (e.g. anti-predator nets, fences, floats, ropes, PVC pipes, and miscellaneous debris), were selected across these regions (see Table 1 for details). Non-aquaculture beaches were chosen to be close enough to shellfish aquaculture sites to minimize differences in oceanography, sediment type, and species assemblages, resulting in locations ~60–530 m from active shellfish aquaculture sites (shortest over-water distance). The Joyce Point site in the Discovery Islands lacked an appropriate reference site within the distances used for the other beach pairs. All sites (shellfish aquaculture and non-aquaculture) were classified according to plastic levels using the following criteria: 'high': hundreds of square metres covered in plastic equipment and/or debris (e.g. large-scale shellfish aquaculture operations that cultured clams and thus had more than 2 synthetic anti-predator nets deployed across the intertidal); 'medium': approximately 10 or more square metres of visible plastic, typically when only 1 or 2 synthetic anti-predator nets or fences and/or several large synthetic debris items such as oyster trays were present; 'low': only scattered plastic items visible (e.g. 1 or 2 ropes, a few scattered pieces

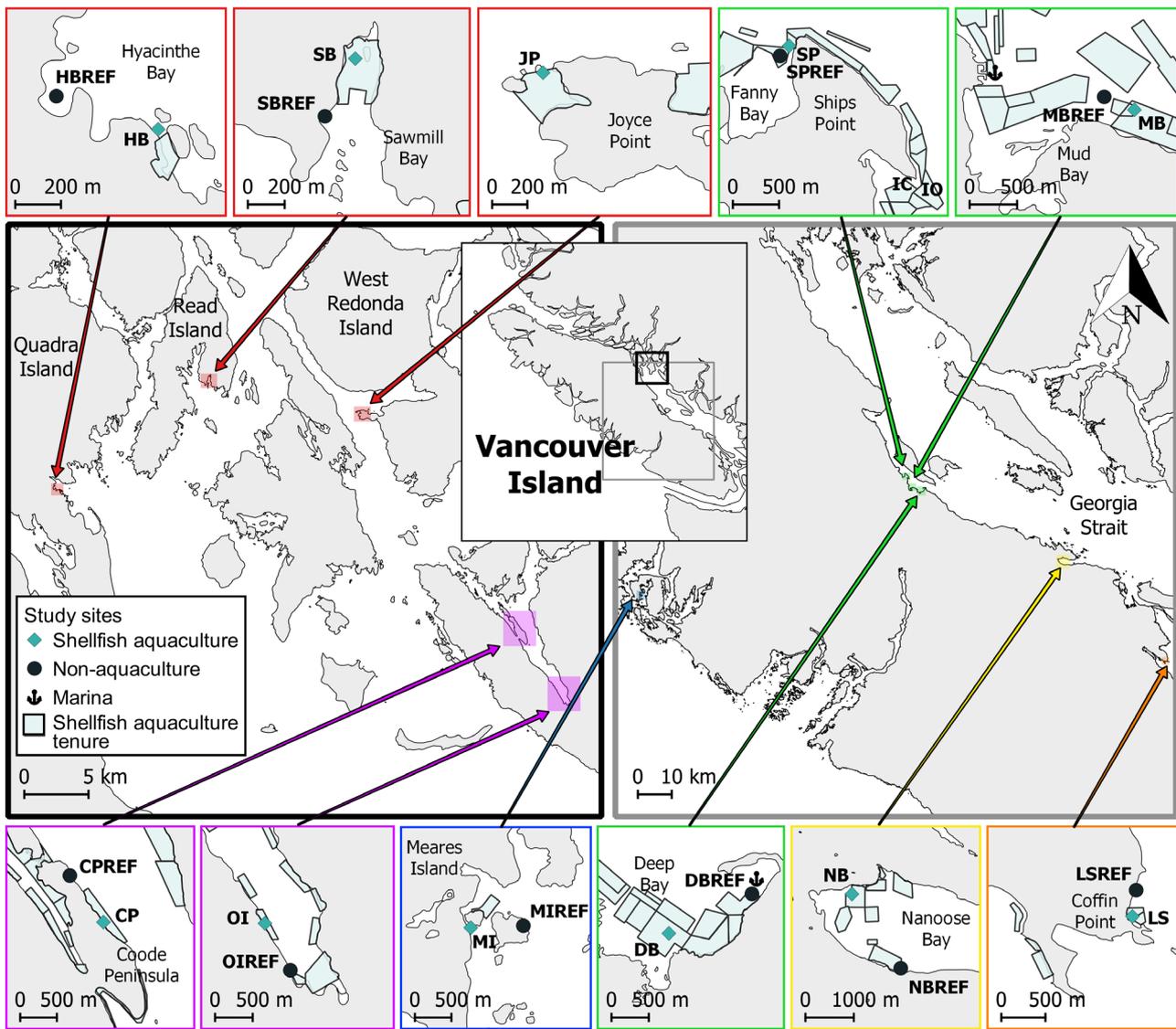


Fig. 1. Study area indicating the 6 regions key to the shellfish aquaculture industry in British Columbia where the study took place. Shellfish aquaculture tenures that have been leased from the government are indicated, but were not all necessarily in active use. Site of initial oyster and clam collection is indicated by IO and IC, respectively, both on the Ships Point panel. Discovery Islands (red) — HB: Hyacinthe Bay, HBREF: Hyacinthe Bay reference, SB: Sawmill Bay, SBREF: Sawmill Bay reference, JP: Joyce Point; Okeover Inlet (purple) — CP: Coode Peninsula, CPREF: Coode Peninsula reference, OI: Okeover Inlet, OIREF: Okeover Inlet reference; Clayoquot Sound (blue) — MI: Meares Island, MIREF: Meares Island reference; Baynes Sound (green) — SP: Ships Point, SPREF: Ships Point reference, MB: Mud Bay, MBREF: Mud Bay reference, DB: Deep Bay, DBREF: Deep Bay reference; NanOOSE Bay (yellow) — NB: NanOOSE Bay, NBREF: NanOOSE Bay reference; Ladysmith (orange) — LS: Ladysmith, LSREF: Ladysmith reference

of debris like car tires, buckets, or bottles); 'none': no plastic visible on site. These classifications were determined from photos taken in each cardinal direction at each point where any type of sampling occurred at every beach. See Fig. 1 for site locations and explanation of site abbreviations.

The Discovery Islands is a low-intensity shellfish aquaculture region spread over hundreds of square kilometres and separated by several islands (Fig. 1).

Okeover Inlet has a moderate density of shellfish aquaculture activity; higher than the Discovery Islands, but not as high as Baynes Sound. Baynes Sound has the highest density of shellfish aquaculture in BC, so all of the reference sites — although being located in gaps between shellfish aquaculture sites — were still within close proximity to a high intensity of both intertidal and deep-water shellfish aquaculture. NanOOSE Bay hosts several shellfish

Table 1. Information on the 21 field sites, including region, species raised, site code used in this paper, sediment type (as determined from average grain-size class proportions from all sediment cores taken at each site and classified according to Wentworth [1922]), degree and types of plastic visible on site, and distance to nearest active shellfish aquaculture site. See Fig. 1 for site abbreviations. Superscript letters after the site codes indicate sample collection and analysis of a: clams, b: oysters, c: water. APN: anti-predator netting; APF: anti-predator fencing; NA: sediment cores not taken; -: no aquaculture

Region	Species raised	Site code	Sediment type	Degree of plastic on site and type	Distance to nearest shellfish aquaculture site (m)
Discovery Islands	Clams and oysters	HB <sup>a,b,c</sup>	Gravelly sand	High: APN	–
	–	HBREF <sup>a,b,c</sup>	Gravelly sand	Low: some ropes and buckets	450
	Clams and oysters	SB <sup>a,b,c</sup>	Gravelly sand	Medium: APN, a car tire	–
	–	SBREF <sup>b,c</sup>	Sandy gravel	Low: 1 balloon ribbon	200
	Oysters	JP <sup>a</sup>	Sandy gravel	Medium: some oyster nets and PVC tubes, floats close offshore	–
Okeover Inlet	Oysters	CP <sup>b,c</sup>	Gravelly sand	Medium: some APF, oyster cages, and PVC tubing	–
	–	CPREF <sup>a,b,c</sup>	Gravelly sand	Low: a rope	240
	Oysters	OI <sup>a,b,c</sup>	Gravelly sand	Medium: APF, PVC tubing	–
	–	OIREF <sup>a,b,c</sup>	Sand	Low: some ropes	500
Nanoose Bay	Clams and oysters	NB <sup>a,b</sup>	NA	High: APN and APF, PVC tubing, ropes, and floats	–
	–	NBREF <sup>a</sup>	NA	None	330
Baynes Sound	Oysters	SP <sup>a,b,c</sup>	Gravelly sand	Medium: APF, some ropes and floats	–
	–	SPREF <sup>a,b,c</sup>	Sand	Low: a boat anchored on the beach	60
	Clams and oysters	MB <sup>a,b,c</sup>	Gravelly sand	High: APN and APF, some PVC tubing	–
	–	MBREF <sup>a,b,c</sup>	Sandy gravel	None	130
	Clams and oysters	DB <sup>a,b,c</sup>	Gravelly sand	High: APN and APF	–
	–	DBREF <sup>b,c</sup>	Gravelly sand	Medium: Several washed up oyster trays and cages and some PVC tubes	250
Ladysmith	Oysters	LS <sup>a,b</sup>	Sand	Medium: Several oyster bags, some ropes and crates, and a car tire	–
	–	LSREF <sup>b</sup>	NA	Low: a single rope	200
Clayoquot Sound	Stocked with oysters, not harvested	MI <sup>a,b,c</sup>	Gravelly sand with silt	Low: some oyster bags and ropes	240
	–	MIREF <sup>a,b,c</sup>	Gravelly sand	None	530

aquaculture operations, as well as recreational collection areas, but with a low degree of plastic use compared to Okeover Inlet and Baynes Sound. At Ladysmith, the aquaculture site was the only shellfish aquaculture operation in the immediate area, although Ladysmith Harbour, a large bay just south of the study area, is a very active industrial area. The Meares Island aquaculture site in Clayoquot Sound

on the west side of Vancouver Island is not technically a harvest site and does not have any plastic infrastructure, but is stocked with adult oysters from a nearby large deep-water operation that utilizes synthetic ropes and large, blue plastic drums as floats. Clayoquot Sound is home to both fish and shellfish aquaculture with an overall density similar to the Discovery Islands.

## 2.2. Bivalve out-plants

A total of 1110 Pacific oysters *Crassostrea gigas* ranging from 74 to 149 mm shell height (mean  $\pm$  SD,  $106.5 \pm 13.6$  mm) were collected on 15 June 2016 from an intertidal shellfish aquaculture site at Ships Point in Baynes Sound (Fig. 1). The oysters were labelled with a black permanent marker and the labels coated with a protective layer of cyanoacrylate glue. The oysters were stored in a flow-through seawater system for 3 to 9 d (unequal times across sites were due to the logistics of transporting oysters to 21 sites over a large geographic area) to facilitate survival during labelling and out-planting to all sites. To test for initial MP contamination, 10 individuals were randomly collected after 9 d and stored at  $-20^{\circ}\text{C}$  until analysis (see Section 3.4).

A total of 1330 Manila clams *Venerupis philippinarum* ranging from 24 to 52 mm shell length (mean  $\pm$  SD =  $37.4 \pm 39.9$  mm) were collected on 17 May 2016 from an intertidal clam culture site at a different location at Ships Point (Fig. 1). A small area on the shell of each clam was sanded down using a rotary tool with sanding attachment (Dremel) and labelled as described above. The clams were then stored in a flow-through seawater system for 2 to 11 d until time of out-planting. Ten individuals were randomly collected after 11 d and stored at  $-20^{\circ}\text{C}$  until analysis for initial MP contamination (see Section 3.4). Although this sampling (in the case of oysters as well) occurred at the end of the deployment period, a difference of several days in the flow-through system is small in comparison to the 3 mo during which the clams were left at the study sites. These initial samples were taken to account for any MPs that had accumulated at the original site and that might therefore still be present at the end of the study.

The clams and oysters were out-planted 22 to 29 May and 18 to 24 June 2016, respectively. Clams were buried at a depth of roughly 2.5 cm at tidal heights of 1.6 to 2.2 m above mean lower low water while oysters were placed on the sediment at 0.9 to 1.9 m tidal heights, depending on the site. Twelve clams and 10 oysters were deployed in  $0.5 \times 0.5$  m quadrats, 10 m apart along each of 2 parallel transects (thus approximating similar tidal heights for each species within a site), totaling 5 quadrats species $^{-1}$  site $^{-1}$  and 60 clams and 50 oysters site $^{-1}$ .

## 2.3. Sample collection

All surviving bivalves that could be found were collected from 22 August to 2 September 2016. Because

of a tendency to gape following freezing, clams were secured shut with natural rubber elastic bands and placed in sealed glass Mason jars for transport. Oysters were wrapped in burlap and sealed in clear-plastic freezer bags for transport. Particle identification later confirmed that the plastic freezer bags did not contaminate the sample. All bivalves were frozen at  $-20^{\circ}\text{C}$  on the day of collection. For statistical purposes, shellfish samples were analyzed if survival was sufficient to allow for the analysis of 10 individuals of a species at each site, resulting in oysters from 19 sites (insufficient survival at Joyce Point and the Nanoose Bay reference site) and clams from 17 sites (insufficient survival at Coode Peninsula and the reference sites for Sawmill Bay, Deep Bay, and Ladysmith) being examined for MPs. In selecting individuals for analysis, and depending on which individuals had survived, bivalves from the first, third, and fifth quadrats along each transect were prioritized to capture site variability and efforts were made to analyze similar numbers of individuals from these quadrats. Any pseudo-replication was accounted for during statistical analysis by the use of hierarchical modeling.

Due to logistical constraints, sediment and water samples were collected and analyzed from only 16 of the 21 sites (excluding Joyce Point, Nanoose Bay, Ladysmith, and the reference sites at Nanoose Bay and Ladysmith), concurrent with shellfish sampling. Sediment cores were collected adjacent to the end and middle quadrats for each species, using 125 ml aluminum corers to a depth of 5.45 cm. This totaled 6 sediment samples site $^{-1}$ , with the exception of both aquaculture and non-aquaculture sites at Sawmill Bay, and the Meares Island aquaculture site where only 5 samples were analyzed for each due to unavoidable sample loss during processing. Half of the samples were collected alongside oysters at 0.9 to 1.9 m and alongside clams at 1.6 to 2.2 m tidal heights.

Water samples were collected by wading to 0.5 m depth during flood tide in a line perpendicular to the shellfish transects at quadrats 1, 3, and 5. At each sampling point, a 1 l sample was taken in a glass Mason jar, totaling 3 samples site $^{-1}$ , although later sample loss resulted in only 1 sample from the aquaculture site at Okeover Inlet and 2 samples each from the aquaculture sites at Hyacinthe Bay and Sawmill Bay being analyzed.

Additional sediment samples for grain-size analysis were collected, using a 5.08 cm diameter PVC corer to a depth of 10 cm, from the middle and both ends of the clam and oyster quadrats, resulting in 3 to

5 samples site<sup>-1</sup> (limited in some cases by bedrock or large cobble). These samples were dried to constant weight, weighed, then separated into 8 grain-size categories ( $\leq 63$ ,  $>63$ – $125$ ,  $>125$ – $250$ ,  $>250$ – $500$ ,  $>500$ – $1000$ ,  $>1000$ – $2000$ ,  $>2000$ – $4750$ , and  $>4750$   $\mu\text{m}$ , adapted from Wentworth [1922]) using stacked sieves and a mechanical shaker. Each fraction was then weighed and the proportion by total sample weight was determined for each size fraction (Eleftheriou 2013). Beach slope was estimated at each site by running a transect line perpendicular to the water's edge (~10–40 m, depending on site), then measuring the vertical displacement with a laser level and calculating the slope angle as  $\arcsin(\text{rise}/\text{run})$ .

#### 2.4. Sample preparation

Shellfish were thawed at room temperature, their shell lengths/heights measured, and all soft tissues removed from shells and dried at 60°C to constant weight. The dry tissues were weighed and then digested for 24 h at 60°C in 10% potassium hydroxide (KOH) (40 ml for clams, 50 ml for oysters). KOH is effective at removing biological material from samples (Foekema et al. 2013, Rochman et al. 2015) and a 24 h incubation at 60°C has little effect on most plastic polymers (Dehaut et al. 2016). Sediment samples were dried at 60°C to constant weight, weighed, and then subjected to overflow flotation using a fully saturated calcium chloride ( $\text{CaCl}_2$ ) solution (density ~1.4 g ml<sup>-1</sup>). Due to the presence of high amounts of largely indigestible plant material, sediment samples were reduced in volume to 1/8 using a Folsom plankton splitter (resulting in a 15.6 ml sample) and then digested with 50 ml of 10% KOH at 60°C for 48 h. Water was removed from the Mason jar samples by securing 8  $\mu\text{m}$  (nominal size) woven, stainless-steel mesh to the mouth of the Mason jar using the metal ring and then inverting the jar over a vacuum filter assembly. Water samples were then dried at 60°C for 48 h or until a constant weight was achieved. Next, 100 ml of 10% KOH solution were added to each sample, which was then covered with aluminum foil and incubated at 60°C for 24 h.

Following the digestion, all samples were vacuum filtered through 47 mm diameter, 8  $\mu\text{m}$  pore size polycarbonate membrane filters (Sterlitech). The glass filter funnels were warmed before filtration (using de-ionized water kept at 60°C) to minimise precipitates forming from the digestate. Filters were then placed in polystyrene PetriSlides (EMD Millipore) for storage and later microscopic visualisation.

All equipment that came into contact with the sample at any time was rinsed thoroughly with filtered de-ionized water. The KOH solution and all de-ionized water used were filtered through 1.6  $\mu\text{m}$  Whatman GF/C filter paper before use.

To minimize background contamination, protocols laid out by Woodall et al. (2015) were adapted. All activity requiring sample exposure to ambient environmental conditions was conducted in a laminar flow hood to reduce contamination from air sources within the laboratory. Blue cotton coveralls and headscarves were worn at all times by laboratory workers to prevent contamination of the samples by synthetic clothing. Blue cotton fibres produced by the coveralls could be readily identified during visual microscopy due to their unique colour and structure. For each day of sample processing, 3 procedural blanks were also processed using identical laboratory methods.

#### 2.5. Visual identification

Visual analysis was conducted for all samples by placing the PetriSlides on a compound microscope stage, removing the cover, and manually scanning the entire sample at 100 $\times$  magnification. To avoid sample contamination at this step, the microscope stage was enclosed in a clear plastic bag which was taped to the bench top and microscope at its edges, as outlined by Torre et al. (2016). MP shape (e.g. fibres, fragments, spherules, films) and colour were noted. Potential microplastic fibres were distinguished from natural fibres according to criteria developed through observation of known synthetic fibres and descriptions from previous studies (e.g. Hidalgo-Ruz et al. 2012). Fibres were identified as suspected MPs if they lacked internal structure, including striations, and were an even width along their length. Each particle was measured along its longest dimension, using cellSens software (Olympus), and assigned to 1 of 6 size categories according to the length of their largest dimension: 10–19, 20–49, 50–99, 100–499, 500–999, and 1000–5000  $\mu\text{m}$ .

#### 2.6. Spectroscopic identification

A sub-sample of the suspected MPs extracted from shellfish and characterized by visual microscopy were identified using Fourier-transform infrared (FTIR) spectroscopy. To select the sub-sample of particles that would be analyzed, 1 each of the clam,

oyster, and water samples from 7 sites (the Deep Bay, Hyacinthe Bay, Mud Bay, and Okeover Inlet aquaculture sites, and the Hyacinthe Bay, Mud Bay, and Okeover Inlet reference sites) were selected randomly. A total of 44 suspected MPs (7 from oysters, 9 from clams, 18 from water, and 10 from sediment samples, all of which were fibres) were analyzed. The particles were lifted from membranes using metal micro-forceps and placed on glass slides that had been coated with a thin layer of 20% dextrose solution for adhesion. Micro-ATR (attenuated total reflectance) FTIR spectroscopy was conducted on a Cary 660 FTIR spectrometer (Agilent Technologies). The infrared signatures of samples taken from aquaculture equipment and plastic debris that were commonly found at the study sites were also examined. Particles were identified using comparisons with the Knowitall spectral library (Bio-Rad Laboratories) of 250 000 entries. Spectral matches were confirmed using quality assurance/quality control as described in Ocean Wise laboratory Standard Operating Procedures.

## 2.7. Data analysis

Particles found in blanks were categorized in the same manner as those from the environmental samples. The average number of particles from each set of 3 blanks (3 sets per round of processing) was rounded up to the nearest integer and subtracted from the counts of each applicable size, colour, and shape category in the corresponding environmental sample (from the same round of processing). This method was used to ensure under- (as opposed to over-) estimation and to maintain a format of positive-integer count data. All corrected concentrations of suspected MPs were then multiplied by a correction factor of 3/22 to account for the average amount of visual identification error as identified by FTIR spectroscopy (discussed in Section 3.3).

All statistical analyses were carried out using R v. 3.5.2 (R Core Team 2015). Generalized linear mixed effect models (GLMMs) were run using the package `glmmADMB` (Fournier et al. 2012, Skaug et al. 2016). GLMMs are useful for capturing the variation within count data, which can only take the form of positive integers and, thus, cannot technically be normally distributed. Error structures approximating zero-inflated Poisson and Poisson distributions and random effects with random intercept and with or without random slope were compared for all GLMMs using second-order Akaike information criterion

( $AIC_c$ ) scores, specialized for small sample size, in the package `MuMIn` (Barton 2018). The best-fitting model (lowest  $AIC_c$ ) was selected on a case-by-case basis. In the GLMMs, 22/3 was used for all samples as an 'offset' term to account for the visual error rate. R uses 'offset' to specify linear predictor variables with a known coefficient of 1, rather than an estimated coefficient. In the case of the Poisson GLMMs used in this study, adding the natural log of these constants is mathematically equivalent to specifying each constant as the denominator for the MP count numbers in the model equation. However, specifying the error factors in this way would fail to satisfy the assumption that Poisson-distributed data only take the form of positive integers. Therefore, the natural log of each constant was used to account for the fact that the other linear predictors are linked to the independent variable (MP count) by a log-link function.

Twenty-two GLMMs were specified according to Table 2. These models were first used to determine whether site location had an effect on MP concentrations in shellfish, seawater, and sediment samples. An offset term of  $\ln(22/3 \times \text{dry-tissue weight})$  was specified for the shellfish models to account for the dry-tissue weight of each individual in addition to the visual identification error rate. Dry weight was used in the offset term rather than as a predictor variable since it did not correlate in any way with MP count for any species, but was still useful as a measure of concentration to compare across individuals. For the oyster data, 1 outlying data point (an oyster from the Hyacinthe Bay reference site with an MP count of 18) was excluded from the analysis to reduce heterogeneity of variance (as visually assessed by residuals vs. fitted values plot). Next, the effect of region on MP concentrations in all sample types was tested. The same outlier was excluded for the oyster model and 1 outlier from the Discovery Islands was excluded from the water sample analysis to improve homogeneity of variance. To account for the fact that the non-aquaculture sites varied in distance from shellfish aquaculture sites, GLMMs were run with distance to nearest shellfish aquaculture site as a predictor for all sample types. Straight-line distance to the nearest shellfish aquaculture site was measured over water using shellfish tenure data from Fisheries and Oceans Canada, GPS coordinates of site locations, and the ruler tool in the QGIS software (QGIS Development Team 2018). One outlier was removed for the sediment model (1 sample from the Sawmill Bay aquaculture site, again assessed visually in a residuals vs. fitted plot). Two GLMMs were also run to determine whether the MP concentrations

Table 2. Generalized linear mixed models (GLMMs) and the response and predictor variables, random effects and whether their slopes and intercepts were assumed to be random or fixed, 'offset' terms specified in R, and the assumed error structure. These models were used in the statistical analyses of variables relating to microplastic particle (MP) concentrations in clams, oysters, seawater, and sediment samples

GLMM	Response variable	Predictor variable(s)	Random effect(s)	Random effect(s) slope/intercept	'Offset' term(s)	Error structure
A	MP count in clams	Site type (shellfish aquaculture/ non-aquaculture)	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
B	MP count in oysters	Site type (shellfish aquaculture/ non-aquaculture)	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
C	MP count in clams	Region	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
D	MP count in oysters	Region	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
E	MP count in clams	Distance to nearest shellfish aquaculture site	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
F	MP count in oysters	Distance to nearest shellfish aquaculture site	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
G	MP concentration in seawater (particles l <sup>-1</sup> )	Site type (shellfish aquaculture/ non-aquaculture)	Site	Fixed slope/ random intercept	Identification error	Zero-inflated Poisson
H	MP concentration in seawater (particles l <sup>-1</sup> )	Region	Site	Fixed slope/ random intercept	Identification error	Poisson
I	MP concentration in seawater (particles l <sup>-1</sup> )	Distance to nearest shellfish aquaculture site	Site	Fixed slope/ random intercept	Identification error	Zero-inflated Poisson
J	MP count in sediment	Site type (shellfish aquaculture/ non-aquaculture)	Site	Fixed slope/ random intercept	Identification error	Zero-inflated Poisson
K	MP count in sediment	Region	Site	Fixed slope/ random intercept	Identification error	Poisson
L	MP count in sediment	Distance to nearest shellfish aquaculture site	Site	Fixed slope/ random intercept	Identification error	Zero-inflated Poisson
M	MP count in clams	MP concentration in seawater (particle l <sup>-1</sup> )	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Poisson
N	MP count in oysters	MP concentration in seawater (particle l <sup>-1</sup> )	Quadrat nested in site	Random slope/ random intercept	Identification error; dry-tissue weight	Poisson
O	MP count in clams	Beach slope (degrees); Grain size PC1; Grain size PC2	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
P	MP count in oysters	Beach slope (degrees); Grain size PC1; Grain size PC2	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
Q	MP concentration in seawater (particles l <sup>-1</sup> )	Beach slope (degrees); Grain size PC1; Grain size PC2	Site	Fixed slope/ random intercept	Identification error	Poisson
R	MP count in sediment	Beach slope (degrees); Grain size PC1; Grain size PC2	Site	Fixed slope/ random intercept	Identification error	Poisson
S	MP count in clams	Amount of plastic on site	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson
T	MP count in sediment	Amount of plastic on site	Site	Fixed slope/ random intercept	Identification error	Poisson
U	MP concentration in seawater (particles l <sup>-1</sup> )	Amount of plastic on site	Site	Fixed slope/ random intercept	Identification error	Poisson
V	MP count in oysters	Amount of plastic on site	Quadrat nested in site	Fixed slope/ random intercept	Identification error; dry-tissue weight	Zero-inflated Poisson

in seawater had an effect on MP concentrations in shellfish.

To approximate the sediment type at each site, an exploratory principal components analysis (PCA) was conducted on the grain-size data using the `prcomp` function in R. PC1 and PC2 accounted for a combined 75.7% of the variance in multi-dimensional space for the grain-size categories (Fig. 2) and so were used as predictor variables in models that appropriately captured the variation in sediment type among beaches. Since grain size, beach slope, and the depositional nature of beaches are known to be correlated (Bascom 1951), separate GLMMs were run for each sample type to determine the effect of beach characteristics on MP concentration (with PC1, PC2, and beach slope used as continuous predictor variables). One outlier from the water samples was excluded from the analysis to improve homogeneity of variance (as visually assessed in a residuals vs. fitted plot).

To test for the impact of plastic presence at any given site on the number of MPs found in shellfish, sediment, and water samples, GLMMs were run with plastic category (high, medium, low, or none) as a

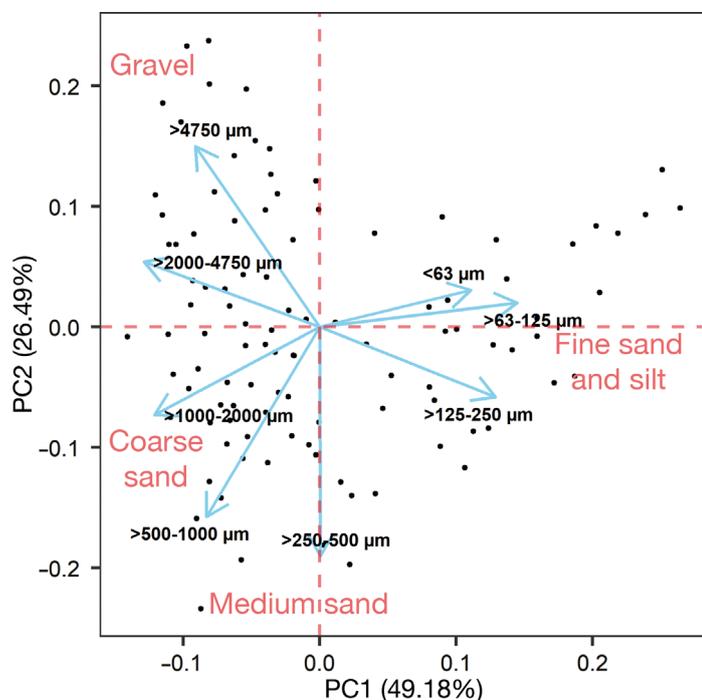


Fig. 2. Principal component analysis results for 8 size classes of sediment grain size across 18 sites. PC1 and PC2 account for 75.7% of the variance with loadings plot indicating which specific grain size classes (in  $\mu\text{m}$ ) accounted for variation along each axis. Sediment size categories according to Wentworth (1922) are indicated to demonstrate how the grain size data grouped in multi-dimensional space

predictor. One outlier was removed for the oyster dataset, as previously specified for the aquaculture vs. non-aquaculture models. For the water dataset, an outlier with an MP concentration of 18 was removed from the high plastic category to reduce heterogeneity of variance. To determine whether plastic category was significant, null models were compared with best-fit models using log-likelihood analysis of deviance tests.

## 2.8. Power analysis

Power analyses using simulation were conducted separately on the clam and oyster data sets. All simulations were run using the package `simr` (Green & MacLeod 2016), which allows statistical power calculations for mixed-effects models through repeated simulation of a given model/experimental design, thereby enabling the user to calculate power curves to assess trade-offs between power and sample size. As the `simr` package requires models to be specified in `lme4` (Bates et al. 2015), the clam and oyster GLMMs (previously used to test for the difference between aquaculture and non-aquaculture sites) were modified to run in `lme4` by removing any zero-inflated error structure and using regular Poisson error structures. For each model, new response data (MP count) were simulated 1000 times and the specified model fit was statistically assessed with each iteration. The calculated power is the proportion of significant test results out of the 1000 iterations at  $\alpha = 0.05$ . For the clam and oyster GLMMs, power curves were calculated to determine the statistical power of each test to detect significance. This was done using a reasonable effect size of  $-1$  for non-aquaculture shellfish (i.e. 1 less MP  $\text{ind.}^{-1}$  in non-aquaculture shellfish than in aquaculture individuals), assuming the pre-existing data structure, but with varying sample size at each site and varying numbers of over-all sites.

## 3. RESULTS

### 3.1. Particle numbers and procedural blanks

Procedural blanks contained (mean  $\pm$  SD)  $0.50 \pm 0.58$  (clams),  $0.37 \pm 0.69$  (oysters),  $4 \pm 2.65$  (water), and  $0.81 \pm 1.36$  (sediment) MPs  $\text{sample}^{-1}$ . A total of 253 (clam), 338 (oyster), 289 (water), and 295 (sediment) potential MPs were identified. After accounting for contamination present in the blanks, total par-

title numbers in the clam, oyster, water, and sediment samples were adjusted to 212 (clams), 310 (oyster), 203 (water), and 254 (sediment).

### 3.2. Particle characteristics

In clams, 90.0% of the remaining suspected MPs (not yet accounting for whether or not they were actually plastic) were fibres, 4.4% were fragments, and 5.6% were spherules. In oysters, the suspected MPs were 90.5% fibres, 2.4% fragments, and 7.1% spherules. Fibres in clams/oysters were predominately clear (37.5%/55.1%), blue (29.5%/16.1%), black (13.5%/10.5%), or turquoise (6.5%/5.6%). The particles of all shapes were primarily 100–499  $\mu\text{m}$  in size for both clams (52.8%) and oysters (49.0%) (Fig. 3). In seawater, 99.5% of particles were fibres, with blue (44.2%), pink (14.2%), turquoise (13.7%), black (12.2%), clear (6.6%), and red (6.1%) being the dominant colours and 1000–5000  $\mu\text{m}$  (38.4%) and 100–499  $\mu\text{m}$  (34.5%) being the dominant size fractions (Fig. 3). Fibres were also dominant in the sediment samples (99.2%) and were mostly clear (46.9%), blue (28.4%), or black (13.0%) and either 100–499  $\mu\text{m}$  (36.6%), 1000–5000  $\mu\text{m}$  (36.2%), or 500–999  $\mu\text{m}$  (27.2%) in length, with no potential MPs <100  $\mu\text{m}$  being detected (Fig. 3).

### 3.3. FTIR analysis

Micro-FTIR analysis confirmed that only 6 of the 44 suspected microplastic fibres (i.e. 13.6%) analysed were actually plastic (Fig. 4). Thus, as described in Section 2.7, a correction factor of 3/22 was applied to all reported suspected MP concentrations. In the 4 clam specimens analyzed, 2 polyester fibres were detected, along with 7 non-plastic fibres. In the 4 oyster samples analyzed, 1 nylon fibre and 1 polyester fibre were detected, along with 5 cellulosic fibres, 1 of which was cotton. From the 5 water samples, 1 polyester fibre, 1 nylon-rayon blend fibre, and 16 non-plastic fibres, including 1 cotton and 1 mineral fibre, were found. In the 3 sediment samples analyzed, all 10 fibres were cellulosic.

Analysis of some of the common plastic items at the study sites determined that anti-predator nets from Mud Bay and Hyacinthe Bay were composed of polypropylene and an unknown polyolefin, and that anti-predator fences used at Mud Bay and Okeover Inlet consisted of polyethylene. Rope debris found on the 2 non-aquaculture sites at Okeover Inlet and Hyacinthe Bay was identified as polypropylene and nylon, while 2 plastic buckets buried in the intertidal at the Hyacinth Bay reference site were both polyethylene. These types of plastic, other than nylon, were not detected in the

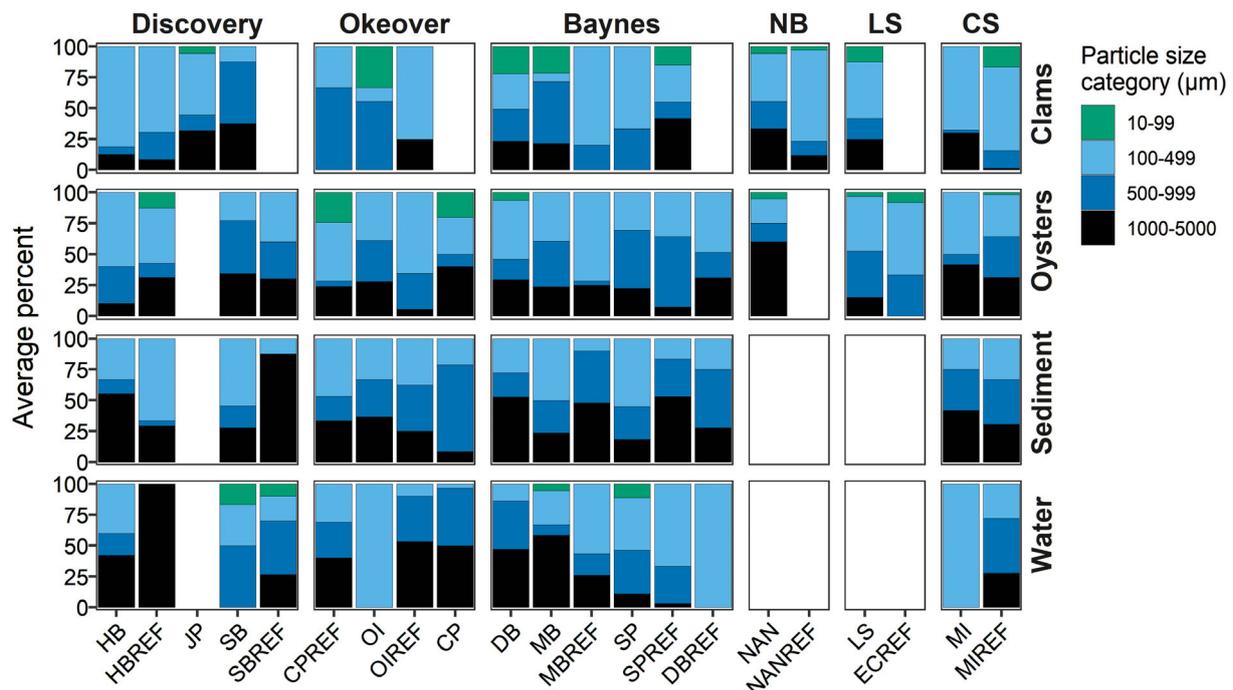


Fig. 3. Average proportion of various potential microplastic particle sizes (longest dimension) of samples that contained these particles in Manila clams *Venerupis philippinarum*, Pacific oysters *Crassostrea gigas*, sediment samples, and seawater samples. Study region is indicated at the top. See Fig. 1 for site and region abbreviations. White spaces indicate where samples from a certain site/type were not analysed

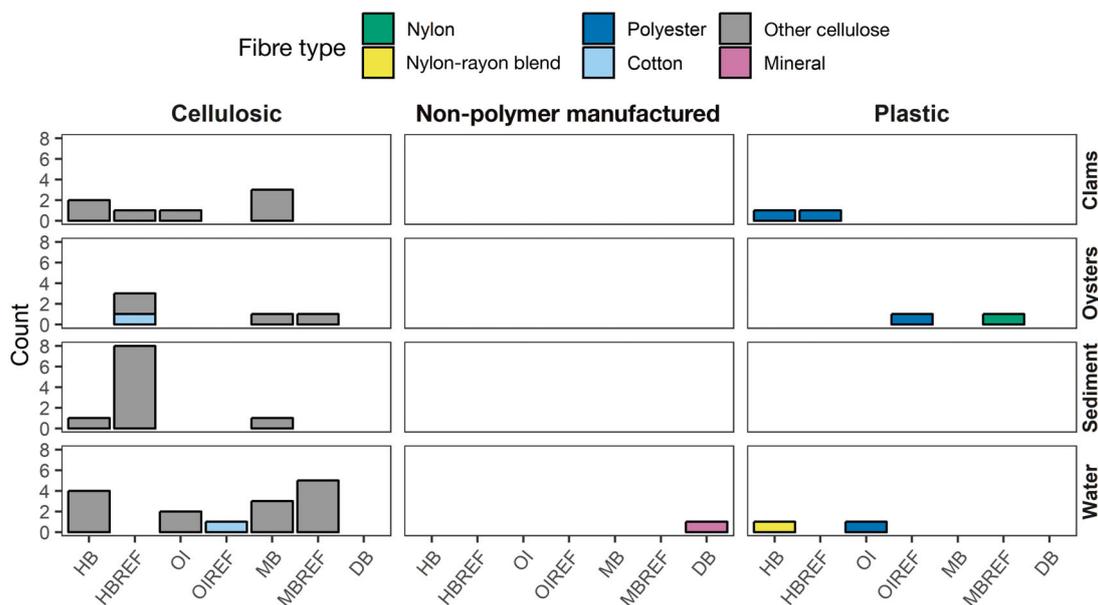


Fig. 4. Fourier-transform infrared (FTIR) spectroscopy results for 44 putative microplastic particles confirming that 6 were plastic and composed of nylon, polyester, or a nylon-rayon blend. Material class is listed across the top and sample type across the right-hand side. Non-polymer manufactured materials include the 1 mineral fibre detected and cellulosic materials cover cotton and all other cellulose-based fibres, including natural (e.g. naturally occurring plant fibres) and semi-synthetic (i.e. rayon and viscose) that could not be reliably separated by FTIR spectroscopy. See Fig. 1 for site abbreviations

synthetic fibres found in clam, oyster, and water samples using FTIR.

### 3.4. MP occurrence

Of the shellfish analyzed for MP content, clams averaged ( $\pm$ SD)  $39.6 \pm 3.5$  mm in shell length and  $0.8 \pm 0.3$  g in dry-tissue weight, while oysters averaged  $108.8 \pm 14.0$  mm in shell height and  $5.7 \pm 2.4$  g in dry-tissue weight. For the initial samples taken before out-plant, clams contained  $0.10 \pm 0.10$  MPs ind.<sup>-1</sup> or  $0.16 \pm 0.18$  MPs g<sup>-1</sup> dry-tissue weight, after adjusting for contamination and identification error, and oysters contained  $0.13 \pm 0.16$  MPs ind.<sup>-1</sup> or  $0.02 \pm 0.03$  g<sup>-1</sup> dry-tissue weight. The clams collected from the out-plant experiment contained a range of ~0 to 2 MPs ind.<sup>-1</sup>, with an average of  $0.16 \pm 0.22$  MPs ind.<sup>-1</sup> or  $0.22 \pm 0.31$  MPs g<sup>-1</sup> dry-tissue weight (Fig. 5). The oysters contained a range of 0 to 3 MPs ind.<sup>-1</sup>, with an average of  $0.22 \pm 0.28$  MPs ind.<sup>-1</sup> or  $0.04 \pm 0.06$  MPs g<sup>-1</sup> dry-tissue weight (Fig. 5). Shellfish out-planted on aquaculture sites did not contain significantly more MPs than those placed at non-aquaculture sites (clams:  $p = 0.37$ ; Table S1A in the Supplement at [www.int-res.com/articles/suppl/q011p357\\_supp.pdf](http://www.int-res.com/articles/suppl/q011p357_supp.pdf); oysters:  $p = 0.56$ ; Table S1B). MP concentrations did not significantly differ among regions for either clams ( $p = 0.06$ ; Table S1C) or oysters ( $p = 0.10$ ; Table S1D).

Distance to the nearest shellfish aquaculture site also had no significant effect on MP concentration in clams ( $p = 0.66$ ; Table S1E, Fig. 6) or oysters ( $p = 0.37$ ; Table S1F, Fig. 6).

The seawater samples contained ~0 to 4 MPs l<sup>-1</sup>, with a mean of  $0.63 \pm 0.68$  MPs l<sup>-1</sup> (Fig. 5), with no significant difference detected between shellfish aquaculture and non-aquaculture samples ( $p = 0.65$ ; Table S1G). Seawater MP concentrations differed significantly by region ( $p = 0.04$ ; Table S1H), with Baynes Sound and Okeover Inlet samples containing more MPs (mean  $\pm$  SD) than those from Discovery Islands and Clayoquot Sounds ( $0.72 \pm 0.57$  and  $0.80 \pm 0.69$  vs.  $0.52 \pm 0.95$  and  $0.25 \pm 0.23$  MPs l<sup>-1</sup>, respectively). Distance to the nearest shellfish aquaculture site had no significant effect on seawater MP concentration ( $p = 0.25$ ; Table S1I, Fig. 6). No significant relationship was detected between MP concentrations in water samples and either clam ( $p = 0.78$ ; Table S1J) or oyster samples ( $p = 0.70$ ; Table S1K) for sites where both shellfish and water samples were taken.

Sediment samples contained ~0 to 3 MPs sample<sup>-1</sup>, with a mean of  $0.37 \pm 0.43$  MPs sample<sup>-1</sup>. This is equivalent to an average of  $23.84 \pm 27.27$  MPs l<sup>-1</sup> or  $23840$  MPs m<sup>-3</sup> (Fig. 5). By dry weight, the average concentration was  $19.97 \pm 23.74$  MPs kg<sup>-1</sup>. No significant difference was detected in sediment MP concentrations between shellfish aquaculture and non-

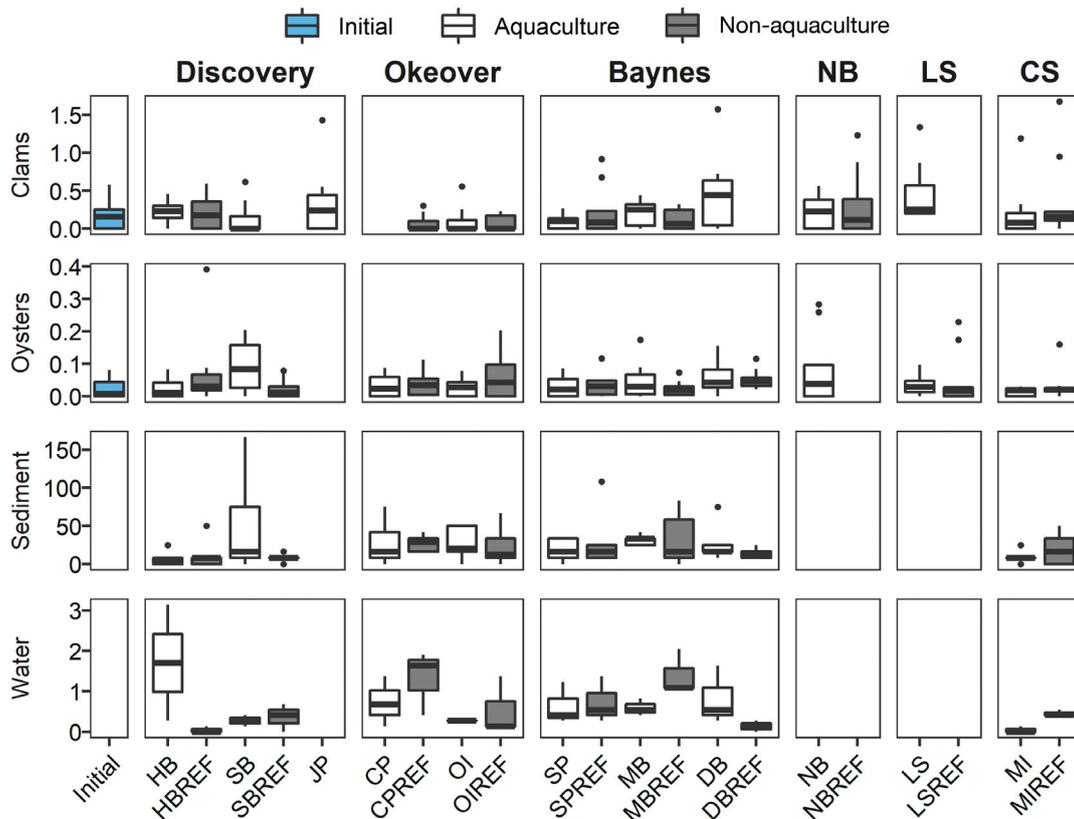


Fig. 5. Boxplots of microplastic particle (MP) concentrations in Manila clams *Venerupis philippinarum* (MP  $g^{-1}$  dry-tissue weight), Pacific oysters *Crassostrea gigas* (MP  $g^{-1}$  dry-tissue weight), sediment samples (MP  $l^{-1}$ ), and seawater samples (MP  $l^{-1}$ ) taken at shellfish aquaculture and non-aquaculture sites. The 'initial' concentrations represent oysters and clams that were sampled before they were out-planted to the various study sites. All concentrations are adjusted for identification error and for background contamination. Box limits and whiskers show first and third quartiles, respectively, with outliers (more than 1.5 times the distance between the first and third quartiles away from the median) shown as points. Study region is indicated at the top, see Fig. 1 for site and region abbreviations

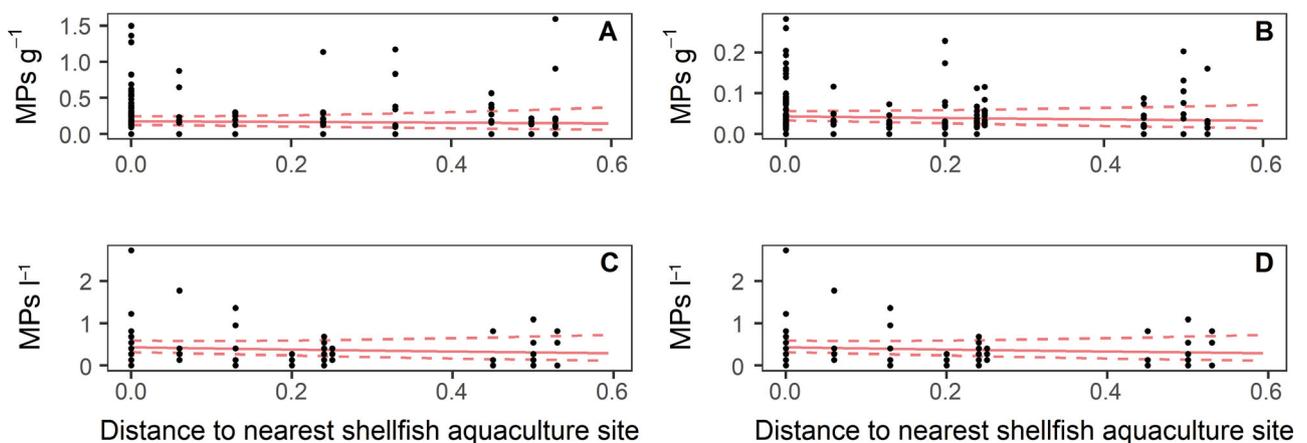


Fig. 6. Microplastic particle (MP) concentrations in (A) clams, (B) oysters, (C) water, and (D) sediment, plotted against shortest overwater distance to nearest shellfish aquaculture site. Concentrations for oysters are in terms of dry-tissue weight. All MP concentrations are adjusted for identification error and for background contamination. The solid red line indicates generalized linear mixed model (GLMM) fit, with random effects set to 0 and dry-tissue weight set to the average value for each of the shellfish species. The dashed red lines indicate the 95% confidence interval for the model fit. Distance to nearest shellfish aquaculture site did not significantly affect MP concentration in any of the models

aquaculture samples ( $p = 0.60$ ; Table S1L). MP concentrations did not differ significantly among regions ( $p = 0.08$ ; Table S1M). Distance to nearest shellfish aquaculture site had no significant effect on sediment MP concentration ( $p = 0.33$ ; Table S1N, Fig. 6).

According to the loadings plot for the grain size PCA (Fig. 2), PC1 differentiates sediments containing more gravel and coarse sand from those with fine sand and silt. PC2 mainly varied according to whether sediment was composed of more sand or gravel and did not vary much according to silt and fine sand composition. No significant effects of beach slope, PC1, or PC2 on MP concentration in clams were detected with the beach characteristics model ( $p = 0.68, 0.23, 0.32$ , respectively; Table S1O). Similarly, beach slope and PC1 were not significant predictors of oyster MP concentration for the beach characteristics model ( $p = 0.84, 0.83$ , respectively; Table S1P). PC2 did have a significant negative effect ( $p = 0.01$ ), suggesting that oysters on sandier beaches may contain more MPs than those on gravel beaches. However, this effect size was extremely small, with only about 0.1 more MPs  $g^{-1}$  dry-tissue weight, on average, predicted at sandier beaches. Beach slope, PC1, and PC2 showed no significant effect on MP concentration in the seawater samples ( $p = 0.64, 0.28, 0.63$ , respectively; Table S1Q) or sediment samples ( $p = 0.28, 0.10, 0.88$ , respectively; Table S1R).

The amount of plastic found on a particular site did not significantly affect MP levels found in clams ( $p = 0.34$ ; Table S1S), sediment ( $p = 0.13$ ; Table S1T), or water samples ( $p = 0.55$ ; Table S1U), but did significantly affect MP levels in oysters ( $p < 0.001$ ; Table S1V). Beaches with no plastic on site had an average of 0.02 less MPs  $g^{-1}$  dry-tissue weight in oysters than beaches with high amounts of plastic on site (0.05 vs. 0.03  $g^{-1}$  dry-tissue weight, on average; Fig. 7). Specifically, this is for the sites DB, MB, NB, and HB (high plastic) compared with MBREF and MIREF (no plastic). However, when considered in terms of individual, the mean number of MPs found in oysters on high vs. low plastic sites are identical (0.20 ind. $^{-1}$ ). Differences according to weight occurred due to the

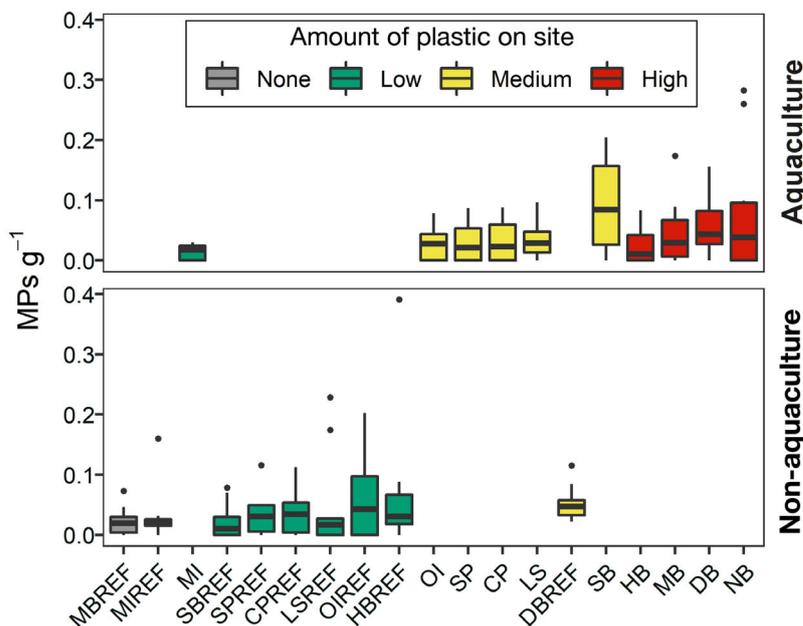


Fig. 7. Boxplots (as defined in Fig. 5) of microplastic particle (MP) concentrations in Pacific oysters *Crassostrea gigas* at 19 study sites (aquaculture and non-aquaculture) according to amount of plastic equipment and debris on site. All concentrations are in terms of dry-tissue weight and are adjusted for identification error and for background contamination. See Fig. 1 for site abbreviations

oysters grown on non-aquaculture sites having higher dry-tissue weights, on average. Comparisons between other levels of plastic impact (high–medium, high–low, medium–low, medium–none, low–none) were not significant (Table S1W).

### 3.5. Power analysis

A power analysis determined that both the clam and oyster data sets were of sufficient power to detect 1 less particle per individual shellfish at a non-aquaculture vs. aquaculture site, given the experimental structure and sample sizes used. The clam analysis had a power of 96% to detect this effect size and would have surpassed a power of 90% with as few as 4 ind. site $^{-1}$ . Further simulation suggests that given the current sample size of 10 clams site $^{-1}$ , as few as 10 total sites (5 of each type) would have been sufficient for 90% power to detect 1 less MP on average in non-aquaculture clams. The oyster analysis had a power of 93% and would have surpassed a power of 90% with as few as 8 ind. site $^{-1}$ . Further simulation suggests that given the current sample size of 10 oysters site $^{-1}$ , as few as 16 total sites would have been sufficient for 90% power to detect 1 less MP on average in non-aquaculture oysters.

#### 4. DISCUSSION

Our results show no significant difference in MP concentrations in shellfish or their habitat between shellfish aquaculture and non-aquaculture sites at a local scale (60–530 m) in coastal BC, suggesting that the observed MP concentrations may be related to other factors. Qualitative assessment of the amount of plastic on each site identified a large variability of plastic use at shellfish aquaculture leases. Oysters from sites with 'high' plastic use, with hundreds of square metres of anti-predator netting, contained significantly more MPs than oysters from non-aquaculture sites with no plastic debris. However, this effect size was small (0.05 vs. 0.03 MPs g<sup>-1</sup> of tissue weight on average). Furthermore, when considered in terms of individual, the average MP concentrations were the same at high and no plastic sites (0.20 ind.<sup>-1</sup>). It can therefore be concluded that the difference in MP concentrations by body weight are driven by higher average tissue weight in the oysters grown at non-aquaculture sites (8.30 vs. 4.29 g dry-tissue) where no macroplastics were present. Spectroscopic results illustrate that the sub-sampled particles identified as plastic were primarily polyester and nylon fibres and that none of the sampled nets were composed of either polymer. The high plastic sites were long, sloping, sandier beaches, which are generally preferred as shellfish aquaculture tenure locations. Sandier beaches were also linked with increased MP concentrations in oysters; however, again this appears to be due to the difference in body size between shellfish aquaculture vs. non-aquaculture sites, as tissue weight was lower at the sandier beaches than the gravellier ones. These findings, along with a lack of correlation between distance to shellfish aquaculture site and MP concentrations, suggests that anthropogenic fibres found in shellfish and their environment in BC are likely not directly linked to shellfish aquaculture operations, but more likely related to textile fibre emissions, the most probable sources of which are sewage effluent and aerial dispersal (Carr 2017). The *in situ* degradation of nylon ropes, which could come from boating, fishing, and/or aquaculture activities, is also a potential source (Welden & Cowie 2017), but none of the materials that were analyzed from the studied shellfish aquaculture operations were actually nylon.

An earlier study in Baynes Sound (one of our study regions) found no difference between Manila clams on/off shellfish aquaculture sites (Davidson & Dudas 2016) and an Italian study found a similar result in mussels (Renzi et al. 2018). In some cases, cultured

bivalves have been documented to contain significantly higher concentrations of MPs than wild shellfish (Mathalon & Hill 2014, Ding et al. 2018, Phuong et al. 2018), but this effect appears to be context dependent. In one case from China, cultured shellfish actually contained significantly fewer MPs than wild individuals when cultured in areas with a comparatively smaller human footprint (Li et al. 2016). Our findings indicate that MP concentrations in bivalves from non-aquaculture sites are similar to those in individuals on shellfish aquaculture sites, despite both site types being distributed across a range of densities of human activity. Our reference sites were only 60 to 530 m from the nearest shellfish aquaculture facility, which makes it difficult to conclusively rule out a contamination effect of shellfish aquaculture that extends beyond such a scale. However, we also did not detect any consistent regional patterns in MP concentrations that would suggest shellfish aquaculture as a source, considering that the density of their activity varied substantially by region.

Previous work has suggested that Baynes Sound is extremely contaminated by small non-fibrous, spherical MPs with up to 25 000 MPs kg<sup>-1</sup> of dry sediment (Kazmiruk et al. 2018), which is 3 orders of magnitude greater than our estimate of 19.97 MPs kg<sup>-1</sup> dry sediment. We found that for the 21 sites examined (in Baynes Sound, Clayoquot Sound, Discovery Islands, southern Vancouver Island, and Okeover Inlet) there was a relatively homogenous distribution of MPs, which conflicts with the suggestion by Kazmiruk et al. (2018) that the high degree of contamination in Baynes Sound is due to the high density of shellfish aquaculture in the region. Interestingly, Kazmiruk et al. (2018) reported the majority of MPs counted to be <0.63 µm using automated photo-identification at 40× magnification (and no FTIR). Given that the lower limit for reliable visual identification of particles has been stated by some researchers as 100 µm in diameter (Dekiff et al. 2014, Lenz et al. 2015), the results of Kazmiruk et al. (2018) are unusual and unprecedented in the published literature.

Most polymers present in the subset of samples analysed using FTIR analyses did not match the composition of equipment used in local intertidal shellfish aquaculture, indicating that other sources likely contributed to the MPs found in our samples. Given the limited size of the FTIR sub-sample, however, this does not necessarily rule out shellfish aquaculture as a source of any of the particles detected. A study in Xiangshan Bay, China found that approximately 56 and 37% of the MPs in seawater and sediment,

respectively, were likely derived from local aquaculture activity, showing that aquaculture can impact the environment with MPs (Chen et al. 2018). In that case, however, the 560 km<sup>2</sup> bay was covered by about 100 km<sup>2</sup> of shellfish and finfish aquaculture activity, a far greater aquaculture coverage than presently occurs anywhere in BC. Thus, while shellfish aquaculture may have the potential to become a significant source of MPs in the environment under highly intensive culture conditions, there is no evidence from our study to suggest that this is currently the case in BC.

The lack of any consistent regional or site patterns in MP concentrations suggests large-scale mixing of MPs across our study area. The majority of our sites were in the Strait of Georgia (SoG), which receives sewage effluent from the Greater Vancouver area as well as from many smaller municipalities along the east coast of Vancouver Island. SoG water is renewed by Pacific Ocean water over a period of several months to a year, depending on depth, and may thus cause homogenization of MPs throughout the entire strait (Pawlowicz et al. 2007). Assuming a minimum sinking rate for fibres of 1 mm s<sup>-1</sup> (calculated for 8 mm fibres, Bagaev et al. 2017), a maximum flow rate of 1.5 m s<sup>-1</sup> in the SoG (from [www.oceannetworks.ca](http://www.oceannetworks.ca)), and subtracting the lowest tidal height of our shellfish samples (0.92 m) from a normal tide range of 3.3 m (Thomson 1981) to assume a maximum depth of 2.38 m for the shellfish to filter feed, we can calculate a maximum sinking time for microfibrils of 40 min and a maximum dispersal distance of 3.6 km. The particles found in this study, however, were much smaller than 8 mm and would thus be subject to micro-scale turbulence effects and low Reynolds number physics, both of which will likely decrease sinking rates, thereby increasing horizontal transport (Bach et al. 2012). Aggregation of MPs with each other and with biogenic particles (Michels et al. 2018) would further complicate the question of sinking rates. Kowalski et al. (2016) estimated sinking rates of 6 to 91 mm s<sup>-1</sup> for plastic pellets (size range: 0.3–3.6 mm) of various polymer types, but the fibres found in our study would again be expected to have slower sinking rates due to their higher aspect ratio. While MPs may be heterogeneous within the SoG to some extent (e.g. near hot-spots such as sewage outfalls), it is more likely that microfibrils remain suspended in the water column over long distances, thereby leading to a more homogenous distribution. This possibility is supported by the ubiquitous presence of MPs in the Arctic Ocean (Lusher et al. 2015) and in the deep sea (Woodall et al. 2014), regions

which would not be expected to receive much direct contamination from anthropogenic sources. Other variables such as temperature (lower temperature, more dense seawater, slower sinking rates), salinity (higher salinity, greater density, slower sinking rates), and aerial transport may further influence the distribution of MPs in coastal BC, and further study of these processes is warranted.

MP concentrations in our sediment samples were 2 orders of magnitude greater than those in our seawater samples, suggesting the eventual deposition and concentration of MPs in sediment. Other studies have also shown that sediment concentrations of MPs can be 1 to 4 orders of magnitude greater than in the overlying water (Woodall et al. 2014, Bagaev et al. 2017). Our estimates, however, should be taken with caution considering that the sediment samples were only ~15.6 ml in volume (after sub-sampling from 150 ml samples) and thus may not be representative of the environment at large. Counting just 1 extra MP particle in such small samples would lead to 2 orders of magnitude change in MP concentration per litre of sediment. As this is similar to the degree by which our sediment and water sample concentrations differed, overestimation of sediment concentration by sampling error cannot be ignored. Furthermore, the subsampled fibres from the sediment were all cellulosic, according to spectroscopy. Our visual error rates may have been much higher for sediment in that case, indicating the presence of many non-plastic fibres in sediment and making it difficult to make any conclusions on MP deposition in our study areas.

The lack of any significant effect of water sample MP concentrations on shellfish or sediment concentrations suggests that the transfer of MPs from seawater to shellfish and sediment is driven at spatial or temporal scales not captured by the instantaneous water sampling used in this study. The particles found in both clams and oysters were both smaller in length than the dominant sizes of MPs found in seawater and sediment, indicating a preference for the capture and ingestion of smaller particles in accordance with the feeding strategies of bivalves (Ward & Shumway 2004). The phytoplankton commonly fed on by shellfish are often less than 100 µm in diameter, but high filtration efficiency has also been demonstrated for chain forming diatoms that can reach up to several hundred µm in length (Nakamura 2001). The low MP concentrations in bivalves (i.e. relative to the finding of 0–4 MPs l<sup>-1</sup> of seawater), along with the predominance of smaller particles, indicates that bivalves may be able to selectively avoid certain MPs, either by avoiding

ingestion or by rejection into pseudofaeces (Defossez & Hawkins 1997), and that the smaller particles that are ingested do not accumulate to any major degree. Clams contained an order of magnitude higher MP concentrations than oysters by tissue weight. This may indicate that clams are more at risk for potential health impacts of MP ingestion than oysters. Pacific oysters have pseudo-lamellibranch gills and can sort particles using both the gills and labial palps (Beninger et al. 2008), while clams have eu-lamellibranch gills, whereby selection function is lowered, which could explain why the clams concentrated MPs more than the oysters. We were not able to reliably measure MPs less than 100  $\mu\text{m}$  in diameter, however, so the absolute concentrations of this size class of particles in the sampled shellfish, and thus the potential for biological risk, remain unknown. Furthermore, this suggests that our concentration estimates for the smaller MPs should be considered conservative underestimates.

While the risk to human health from ingesting MPs via the consumption of shellfish has not yet been quantified, it will likely be low compared to other exposure pathways, given the low consumption of shellfish relative to other food items (in many cultures) and the magnitude of other exposure vectors. We found means of  $0.22 \pm 0.28$  MPs ind.<sup>-1</sup> or  $0.04 \pm 0.06$  MPs g<sup>-1</sup> dry-tissue weight in Pacific oysters. Other studies have reported averages for the same species of 30 and 87 MPs g<sup>-1</sup> dry weight from 2 locations on the Dutch coast (Leslie et al. 2017), 2.1 MPs oyster<sup>-1</sup> on the French Atlantic coast (Phuong et al. 2018), and 0.6 MPs oyster<sup>-1</sup> on the US. Pacific coast (Rochman et al. 2015). Our numbers are much lower than oysters sampled in Europe, but similar to those examined in the USA. We found  $0.16 \pm 0.22$  MPs ind.<sup>-1</sup> in Manila clams, while a previous study conducted in one of our experimental regions, Baynes Sound, reported 11.3 and 8.4 MPs clam<sup>-1</sup> on/off shellfish aquaculture sites (Davidson & Dudas 2016) and a study in China reported 5.7 MPs clam<sup>-1</sup> (Li et al. 2015). The lower levels in the present study than in Davidson & Dudas (2016) are most likely due to no FTIR-adjustment in the latter. This is a common problem in microplastics research as the methodology is rapidly evolving, rendering it difficult to make comparisons with older studies or other regions using different experimental methods.

Statistics Canada (2018) reported that 1.58 kg of shellfish person<sup>-1</sup> (not adjusted for losses such as waste and/or spoilage) was available for consumption by Canadians in 2017. Assuming that this amount of shellfish was actually consumed by the average

Canadian, and that 50% consisted of Manila clams and 50% of Pacific oysters, with wet weight:dry weight ratios of 2 and 5 for Manila clams and Pacific oysters, respectively (Mo & Neilson 1994, Yang et al. 2010), our findings suggest that per capita Canadian consumption of MPs via shellfish could be as low as 87 particles person<sup>-1</sup> yr<sup>-1</sup>. This is similar to an estimate of 123 particles per person<sup>-1</sup> yr<sup>-1</sup> in the UK generated by Catarino et al. (2018), who further noted that fibre exposure during evening meals could lead to consumption of 13 731–68 415 particles person<sup>-1</sup> yr<sup>-1</sup>. It is therefore important to highlight that although MPs undoubtedly are found in shellfish, the risk of exposure to humans via their consumption can likely be considered negligible when compared with other sources of MP exposure.

## 5. CONCLUSIONS

Our findings suggest that shellfish aquaculture is not currently a major source of microplastics in BC waters. While this does not mean that shellfish aquaculture equipment is not releasing MPs into the environment from the plastic used in its operations, it does indicate that other sources of MPs (e.g. textile emissions via sewage or aerial dispersal) may be dominating over any local signal from shellfish aquaculture. Significantly (albeit minimally) lower MP concentrations by dry-tissue weight in oysters grown on more gravelly beaches with no macroplastics present (i.e. non-aquaculture sites) appear to be caused by greater tissue weight in these oysters, suggesting that the oysters did not ingest fewer MPs on the level of the individual. If precautionary best practices to minimize microplastic contamination are employed by shellfish farmers, such efforts would be best targeted towards removal of or alternatives for fibrous equipment like ropes and anti-predator nets which can photodegrade and potentially release large amounts of synthetic fibres into the environment.

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