

Microplastic Ingestion by Wild and Cultured Manila Clams (*Venerupis philippinarum*) from Baynes Sound, British Columbia

Katie Davidson^{1,2} · Sarah E. Dudas^{1,2}

Received: 19 January 2016 / Accepted: 9 May 2016 / Published online: 3 June 2016
© Springer Science+Business Media New York 2016

Abstract Microplastics, plastic particles <5 mm, are an emerging concern in aquatic ecosystems. Because microplastics are small, they are available to many filter-feeding organisms, which can then be consumed by higher trophic level organisms, including humans. This study documents the quantity of microplastics present in wild and cultured Manila clams (*Venerupis philippinarum*). Three active shellfish farms and three reference beaches (i.e., non-shellfish farm sites) in Baynes Sound, British Columbia were chosen to examine the microplastic concentrations in wild and cultured Manila clams. Microplastics were isolated using a nitric acid digestion technique and enumerated from 54 clams (27 farmed and 27 non-farmed). Qualitative attributes, such as colour and microplastic type (fiber, fragment, or film) also were recorded. There was no significant difference ($F = 1.29$; $df = 1,4$; $P = 0.289$) between microplastic concentrations in cultured and wild clams. Microplastic concentrations ranged from 0.07 to 5.47 particles/g (from reference beach and shellfish farm clams, respectively). Fibers were the dominant microplastic (90 %); colourless and dark gray fibers were the most common colours observed (36 and 26 %, respectively). Although this indicates that microplastics are definitely present in seafood consumed by humans, shellfish aquaculture operations do not appear to be increasing microplastic concentrations in farmed clams in this region.

Microplastics are an emerging pollutant with the potential to affect many trophic levels. The definition of what constitutes a microplastic varies in the literature, including any plastic fragment <1 mm (Browne et al. 2008, 2011) or <5 mm (Arthur et al. 2009; Fendall and Sewell 2009; Hidalgo-Ruz et al. 2012; Eerkes-Medrano et al. 2015; Rocha-Santos and Duarte 2015). Although these particles can be microscopic, they can persist in the environment for many years (Moore 2008; Rios et al. 2007). There are two types of microplastics, which are distinguished by their sources (Wright et al. 2013b; Duis and Coors 2016). Primary microplastics enter the environment as microscopic plastic particles (e.g., microbeads in facial cleansers, fibers from washing synthetic clothing) (Fendall and Sewell 2009; Browne et al. 2011; Cole et al. 2011). Secondary microplastics enter the environment as larger macroplastics (e.g., plastic water bottles, polyethylene bags, fishing gear, etc.) and become microplastics through environmental degradation into progressively smaller particles (Rios et al. 2007; Andrady 2011).

Unfortunately, microplastics are not limited to a particular area of the world or ecosystem (Rocha-Santos and Duarte 2015). They have been documented along shorelines around the world on all human-habited continents (Browne et al. 2011) and are present in sediments (Browne et al. 2011; Stolte et al. 2015), soils (Zubris and Richards 2005; Rocha-Santos and Duarte 2015), and the water column of marine (Desforges et al. 2014; Mathalon and Hill 2014; Desforges et al. 2015) and freshwater (Eriksen et al. 2013; Lechner et al. 2014; McCormick 2015) ecosystems. The ability for microplastics to sorb, concentrate (Mato et al. 2001; Endo et al. 2005; Ogata et al. 2009), and release (Teuten et al. 2007, 2009) manufacturing chemicals and naturally occurring environmental pollutants to organisms, followed by their bioavailability to a variety of organisms

Sarah E. Dudas is designated as a shared first authorship.

✉ Sarah E. Dudas
Sarah.Dudas@viu.ca

¹ Biology Department, Vancouver Island University, 500 Fifth St., Nanaimo, BC, Canada

² Centre for Shellfish Research, Vancouver Island University, 500 Fifth St., Nanaimo, BC, Canada

(Rocha-Santos and Duarte 2015), makes them a serious emerging threat to wildlife and natural ecosystems and potentially human health.

In particular, microplastics are well-documented in the marine environment. They can be ingested naturally by a variety of organisms with different feeding mechanisms, including zooplankton (Desforges et al. 2015), filter-feeding bivalves (Mathalon and Hill 2014; Van Cauwenberghe et al. 2015), detritivorous lugworms (Van Cauwenberghe et al. 2015), fish (Boerger et al. 2010; Lusher et al. 2013), and even humpback whales (Besseling et al. 2015). Ingestion of microplastics can have negative effects due to chemical or physical damage of the digestive tract (von Moos et al. 2012; Wright et al. 2013a; Avio et al. 2015; Cole et al. 2015; Nobre et al. 2015). This is a concern for organisms and the implications for their roles in food webs but also possibly for economically important species, particularly those cultured for human consumption.

Shellfish are ecologically and economically important. They are prey for many vertebrate and invertebrate consumers and are a primary food source cultured for human consumption around the world. In Canada, the aquaculture industry is valued at around \$962 million (Fisheries and Oceans Canada 2013). Cultured shellfish represent 10 % of the value of the aquaculture industry in Canada and 24 % of the mass of cultured organisms (Fisheries and Oceans Canada 2014). It includes blue mussels (*Mytilus* spp., 3 species), clams (6 genera), and oysters (*Crassostrea* spp., 2 species). On the east coast, mussels are the dominant species of culture, whereas the west coast hosts more clam and oyster farms (Fisheries and Oceans Canada 2015). Both shellfish and finfish aquaculture operations may contribute to microplastic presence in the marine environment (Desforges et al. 2014; Hinojosa and Thiel 2009) through the use of plastic infrastructure. Specifically, it has been speculated that shellfish aquaculture operations may be point sources of microplastics on the west coast of Canada due to the use of plastic ropes, floats, and nets and due to the industry's rapid increase in recent years (Bendell 2015).

Despite this concern, few studies have compared microplastic concentrations between cultured and wild aquatic organisms. Microplastic loads specifically in cultured mussels have been relatively well-documented in Europe (Van Cauwenberghe and Janssen 2014; Vandermeersch et al. 2015) and China (Li et al. 2015), but only a few studies have explicitly compared concentrations between cultured and wild bivalves (De Witte et al. 2014; Mathalon and Hill 2014; Vandermeersch et al. 2015). Mussels (*Mytilus* sp.) have been the main focus of these studies, and documentation about the microplastic concentrations in other cultured species is lacking.

We collected a commonly farmed species, the Manila clam (*Venerupis philippinarum*) directly from shellfish

aquaculture leases and “reference beaches” (i.e., non-farm sites) to determine: (a) if microplastics were present in these clams on the west coast of Canada, and (b) if difference exists in microplastic concentrations between farmed and wild clams. These sites were located in Baynes Sound on the east coast of Vancouver Island, British Columbia, which is vital to Canada's shellfish aquaculture economy. Furthermore, it has been suggested as an area in need of study due to the ubiquitous use of plastic by the shellfish industry (Bendell 2015). This water body hosts approximately one-half of the shellfish farm leases in British Columbia within a ~20-km stretch of sheltered water and is an economically and ecologically important area (Jamieson et al. 2001). We predicted that farmed clams, which may be exposed to more plastics on farm sites due to plastic farm infrastructure, would have higher levels of microplastics than non-farmed clams.

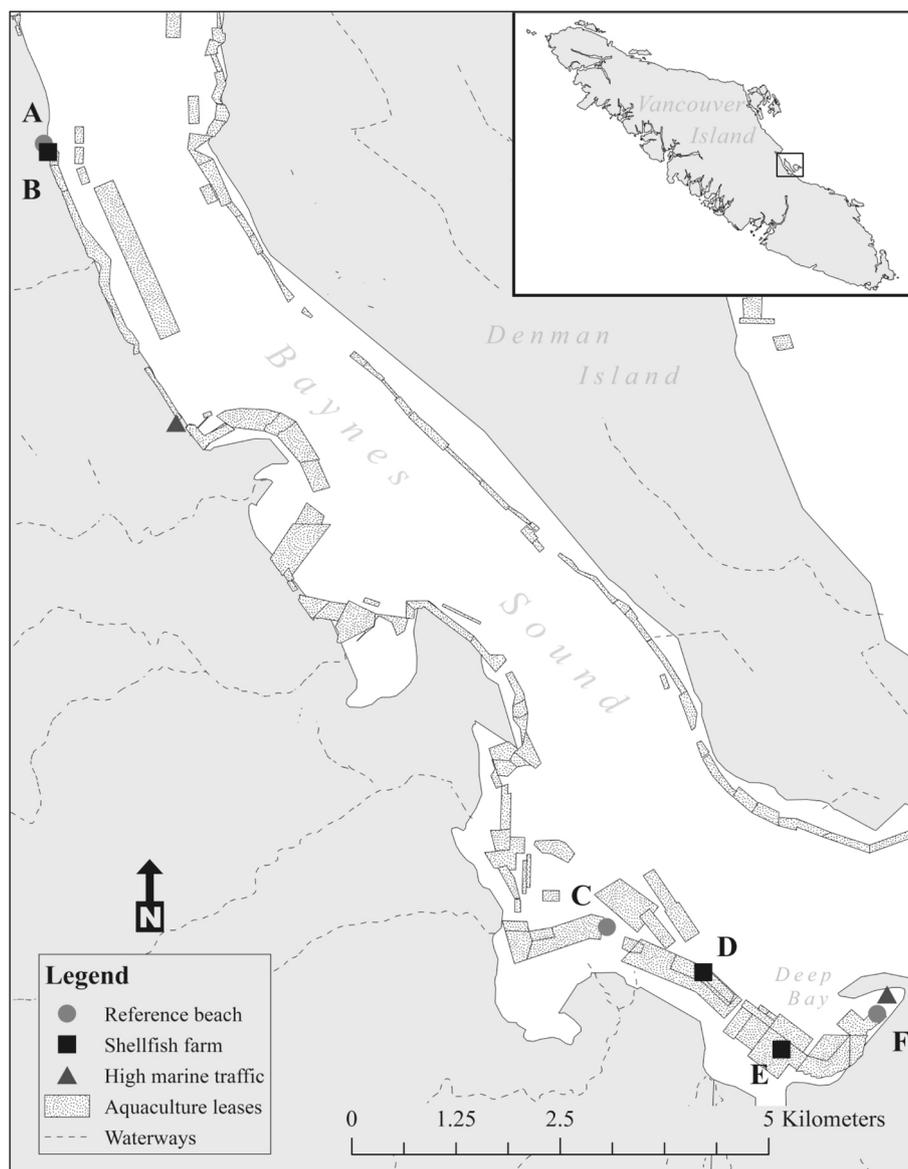
Materials and Methods

Study Area

Baynes Sound is a 20-km long body of water located between Vancouver Island and Denman Island, British Columbia that consists of protected bays, open shoreline, estuaries, inshore marshes, and forests (Jamieson et al. 2001). Tidal flushing occurs between flood and ebb tide with the net circulation of water flowing from north to south; the water movement is primarily tidal with occasional wind-influenced currents (Jamieson et al. 2001). Furthermore, there is evidence of retentive circulation of water in Deep Bay (S. Dudas, personal communication). It also supports an extensive shellfish farming industry, with more than 100 individual leases in the sound on the east shoreline of Vancouver Island and west shoreline of Denman Island (Fig. 1). The industry primarily grows Pacific oysters (*Crassostrea gigas*) and Manila clams (*Venerupis philippinarum*).

There are several potential microplastic sources in Baynes Sound. Oyster farming involves the use of polypropylene fencing, trays, and rope (some rope may be nylon), whereas clam farming uses anti-predator netting made of nylon and/or polypropylene. Floats that support oyster trays and ropes are mostly made of polystyrene. Other potential local sources of microplastics may include recreational boat traffic (including recreational fishing gear that uses plastic fishing line, floats, etc.) and boats in marinas (including the Deep Bay marina and ferry terminals), which release bilge water and may use microplastics in cleaners or for “sand blasting” boats. There also is a possibility for microplastic inputs from residential wastewater; although the majority of the systems are septic,

Fig. 1 Baynes Sound with six sampling sites, three shellfish farms (square) and three reference beaches (circle). Note that the reference beach at site F is currently an inactive shellfish farm lease. High marine traffic areas (triangles) indicate the ferry (north) and marina (south)



many are failing (CVRD 2015) and may be releasing unfiltered residential wastewater into the marine environment. There also is a wastewater outfall site located north of the Sound in Comox (K. Garrett, CVRD personal communication); although the net movement of water in Baynes Sound is from north to south (Jamieson et al. 2001), it is likely that the effluent would bypass the Sound completely because it is located 3 km offshore (K. Garrett, CVRD personal communication).

Spatial Data Acquisition

Spatial data was obtained from several sources. Basic coastline data, waterways, and ocean layers were obtained from Newton & Gilchrist (2010). Comox Valley Regional

District (CVRD) property parcels (current to 2012) and terrestrial zoning (current to 2005) were obtained from the CVRD Public Affairs and Information Systems Branch (T. Hardy, personal communication). Regional District of Nanaimo (RDN) property parcel layers were obtained from the RDN GIS Coordinator (T. Sohler, personal communication). Shellfish farm leases (“TANTALIS—Crown Tenures,” created 2008 but updated constantly; C. Osborne, BC Government, personal communication) were downloaded from the BC Provincial Government DataBC website (<http://www.data.gov.bc.ca>). Shellfish farm and reference beach coordinates were obtained using a hand-held GPS unit (Garmin eTrex30, ~10 m precision), and coordinates for “high marine traffic locations” were obtained from Google Earth (v 7.1.2.2041). ESRI

ArcMap (v. 10.2.2.3552, 2014) was used for all spatial analysis.

Sample Collection

Clams were collected from six sites: three active shellfish farms (sites B, D, E) and three reference beaches currently unused by the shellfish farming industry (sites A, C, F) along the east coast of Vancouver Island in Baynes Sound (Fig. 1). At shellfish farms sites, anti-predator netting was pulled back and clams were obtained from directly underneath the nets. At reference sites, a random location on the beach was chosen; sampling occurred at an average tidal height of $2.42 \text{ m} \pm 0.24 \text{ m}$ at all sites. Clams were obtained using a survey method adapted from the Fisheries and Oceans Canada clam survey manual, whereby Manila clams were obtained using a $0.5\text{-m} \times 0.5\text{-m}$ quadrat to a depth of 20 cm or greater (Gillespie and Kronlund 1999). At each site, a 17-m transect was laid parallel to the water line and three quadrats were evenly spaced approximately 5-m apart. Quadrats were excavated using a hand trowel and the first three clams of approximately equal size (40–45 mm length) were retained. A total of 9 clams were retained from each site, resulting in 54 clams from six sites (27 clams from three shellfish farms, 27 clams from three reference sites).

Clams were refrigerated ($1 \text{ }^\circ\text{C}$) before and during preliminary processing, at which time clams were rinsed and shell length (lateral length), width (umbo to edge), and height (depth of shell) were measured using calipers (0.5-mm precision). Clams were then frozen until acid digestion could take place. During freezing clams gape, creating a potential route for contamination. To avoid this, clams were bound with elastic bands while in the freezer to maintain pressure on both sides of the shell.

Following similar protocols in other studies (Mathalon and Hill 2014; Li et al. 2015), clams were not depurated as we wished to include microplastics that had been both recently ingested and possibly translocated to tissues. Typically bivalves are depurated to avoid dealing with sand particles that may affect the method of processing (Claessens et al. 2013; Van Cauwenberghe et al. 2015) or because animals were being prepared for use in feeding trials (Browne et al. 2008; von Moos et al. 2012; Avio et al. 2015). We would expect that microplastic concentrations reported would be slightly lower if clams had been depurated (Van Cauwenberghe and Janssen 2014).

Acid Digestion and Microplastic Identification

Prior to acid digestion, clam shells were rinsed thoroughly with water and shelled weights were obtained using a digital scale (Sartorius CP124S, 0.0001 g precision). Clam

shells were then removed using a scalpel and forceps, and the soft tissue was weighed separately. Clam tissue was then digested in 40 mL of 69–71 % nitric acid (HNO_3 , ACS grade A200-212, Fisher Scientific) for 4 h in a hot water bath ($\sim 90 \text{ }^\circ\text{C}$) using methods adapted from Claessens et al. (2013) and Desforges et al. (personal communication). Digestions were considered complete when there was no visible organic material remaining and the solution was clear and yellow. The digested solution was then diluted (1:10) with warm ($\sim 90 \text{ }^\circ\text{C}$) deionized water and vacuum filtered over $1.2\text{-}\mu\text{m}$ glass microfiber filters (Whatman GF/C). All beakers and Buchner funnel were rinsed with deionized water before digestion and throughout to reduce the amount of sample lost during transfer stages. Filter paper was then examined under a dissecting scope (10–40 \times magnification) for microplastic particles. When necessary, individual microplastic fragments were further examined under a compound microscope (10–100 \times magnification). Microplastic abundance, colour, and shape (fiber, fragment, or film) were recorded.

Microplastics were identified by the characteristics outlined in Desforges et al. (2014) (M. Galbraith and P. Ross, personal communication), such as flexibility, colour, structure, and lack of biological features. For example, glass fibers from filter paper appear dense and smooth under the microscope (Fig. 2a) and break easily into shards or geometric fragments. After nitric acid digestion, plastics retain colours that are not normally observed in the environment, for example, bright shades of red, blue, green, or yellow. Sand and glass were identified as nonplastic due to their geometric or tetrahedral appearance (they often have distinct edges and squared corners). Microplastics also tend to be frayed and folded over, and fibers are never perfectly straight and rigid (as glass fibers tend to be) (Fig. 2b).

Data Analysis

Values were expressed as the mean number of microplastic particles per 1 g of clam tissue to standardize for clam size (“particles/g”). A nested analysis of variance (ANOVA; $\alpha = 0.05$) was used to compare microplastic concentrations found in farmed and non-farmed clams from each of the six sites, “site” (A–F) nested within “class” (“farm” vs. “wild”) using the *aov* function in RStudio (v 0.98.1091). Characteristics, such as colour and type (fiber, film, or fragment) were also recorded during processing for qualitative comparisons. The most common microplastic type was then analyzed further using a Chi square test of association ($\alpha = 0.05$) to determine if a relationship between fiber colour and “class” (“farm” vs. “non-farm”) existed using the *chisq.test* function in RStudio (v 0.98.1091). Qualitative characteristics were then expressed

Fig. 2 a Glass fiber isolated from filter paper (compound microscope, 10× magnification; scale bar represents 2.5 mm). **b** Microplastic fiber from Manila clam exhibiting fraying (arrows) (compound microscope, 40× magnification)



as frequency data (i.e., percentages) for overall comparisons. All values are reported as mean \pm standard deviation ($\bar{x} \pm SD$).

Procedural Blanks and Contamination Mitigation

To mitigate airborne and lab contamination, cotton clothing was worn throughout lab processing and metal and glassware were used as much as possible. Some plastic could not be avoided, such as plastic piping in the water distillation apparatus, but all glassware and tools were thoroughly rinsed with deionized water throughout processing and between clam digestions. All beakers were covered with tinfoil to reduce air-borne microplastic contamination. Five procedural blanks also were run using the previously mentioned method, which ensured the nitric acid was not contaminated and exhibited the level of airborne contamination present in samples. Filter papers were left out in the fume

hood and on countertops near processing to determine “hot spots” of airborne contamination.

Several clams and blank samples were spiked with polyethylene beads (from a facial cleanser) and polystyrene spheres (approximately 2–3 mm diameter) and were digested in nitric acid using the same method previously mentioned. Microscopic analysis indicated that the nitric acid digestion had little-to-no impact on the integrity of the polyethylene beads; polystyrene spheres shrunk in size considerably but were still visible with the un-aided eye.

Results

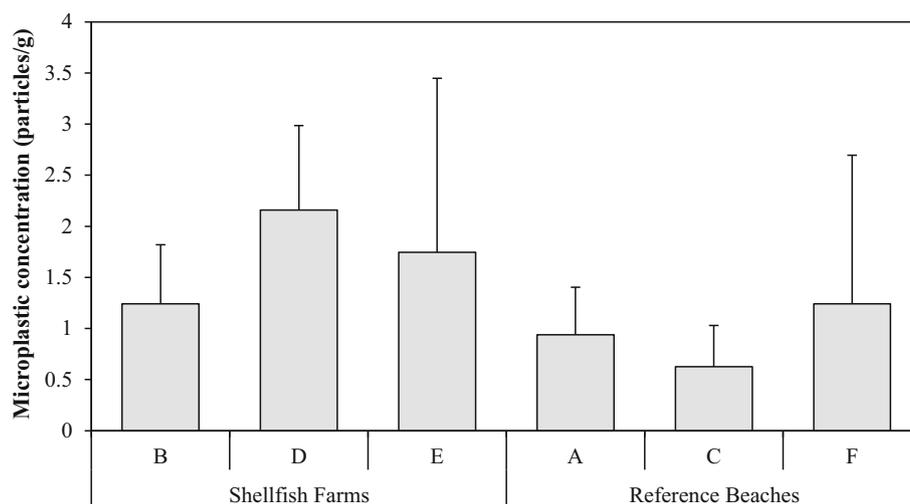
Wild Manila clams were larger and heavier than cultured clams, although some variation between sites occurred (Table 1). All weights reported from here on are soft tissue weights only.

Table 1 Mean ($\pm SD$) weights (g), sizes (mm) and observed microplastic concentrations (particles/clam) at each study site and across all farmed and wild Manila clams sampled

Source	Site	Mass (g)		Measurement (mm)			Microplastic concentration
		Soft tissue	Shelled clam	Length	Width	Depth	Particles/clam
Wild		10.0 \pm 3.8	23.4 \pm 9.2	44.6 \pm 5.2	36.2 \pm 3.9	24.0 \pm 3.2	8.4 \pm 8.5
	A	7.1 \pm 2.0	16.8 \pm 5.4	41.0 \pm 3.5	32.9 \pm 2.6	21.7 \pm 2.2	6.1 \pm 2.5
	C	10.1 \pm 2.5	23.2 \pm 7.0	46.1 \pm 4.0	36.7 \pm 3.7	23.4 \pm 2.6	6.4 \pm 5.7
	F	13.0 \pm 4.3	30.1 \pm 10.3	46.7 \pm 6.4	38.9 \pm 3.3	26.8 \pm 2.8	12.7 \pm 13.0
Farmed		7.0 \pm 1.5	16.6 \pm 3.5	40.7 \pm 2.8	31.9 \pm 2.6	21.1 \pm 1.8	11.3 \pm 6.6
	D	7.4 \pm 1.6	16.6 \pm 3.3	41.6 \pm 3.7	31.3 \pm 1.6	20.6 \pm 1.3	15.4 \pm 6.3
	E	6.0 \pm 1.0	14.9 \pm 1.5	39.0 \pm 1.2	30.4 \pm 1.4	20.4 \pm 0.7	9.3 \pm 7.3
	B	7.5 \pm 1.5	18.5 \pm 4.5	41.6 \pm 2.6	34.1 \pm 3.3	22.2 \pm 2.5	9.1 \pm 4.3

Length refers to the distance from anterior to posterior; width refers to the distance from umbo to edge; depth to the “thickness” of the clam between right and left shells

Fig. 3 Microplastic concentrations (mean number of particles/g \pm SD) in farmed (B, D, E) and wild (A, C, F) Manila clams from Baynes Sound, BC



Comparison of Farmed and Non-farmed Clams

There was no significant difference in microplastic concentrations between cultured clams (1.7 ± 1.2 particles/g) and wild clams (0.9 ± 0.9 particles/g) ($F = 1.29$; $df = 1,4$; $P = 0.289$). Clams from site D, a shellfish farm, exhibited the highest concentration of microplastics (2.2 ± 0.8 particles/g), whereas site C clams, from a reference beach, exhibited the lowest concentration (0.6 ± 0.4 particles/g; Fig. 3). We also report concentrations as number of particles per clam for comparison to external contamination recorded during processing of procedural blanks and for comparison to other studies (Table 1).

Microplastic Fibers

Fibers were the most commonly found type of microplastics in Manila clams (90 %), followed by films (5.3 %) and fragments (4.7 %). Clams from shellfish farms had more fibers (59 %) than clams from reference beaches (41 %). Of all the fibers recorded within farmed clams, colourless, gray, and yellow fibers were the dominant colours; in non-farmed clams, colourless, red, and yellow fibers were dominant. Of all the microplastic fiber colours found, green, gray, and colourless fibers occurred more frequently in clams from shellfish farms, whereas red, blue, and purple fibers occurred more frequently in clams from reference beaches. Yellow fibers occurred in equal frequency at both shellfish farms and reference beaches. Overall, there was a significant relationship ($\chi^2 = 60.3$, $df = 6$, $P < 0.001$) between fiber colour and site type (farm vs. reference beach). Colourless fibers accounted for 36 % of the total fiber colours noted, followed by gray fibers (26 %). The

least common fiber colours were green (5 %) and purple (2 %).

Procedural Blanks (Controls)

To determine the extent of microplastic contamination that could be attributed to processing the samples and external contamination, five procedural blanks were run through the complete digestion and vacuum filtration procedure. The average concentration of microplastic contamination was 5.8 ± 2.2 particles/sample (range 3–8 particles/sample). Again, fibers were the most common (83 %; $n = 24$), followed by films (14 %; $n = 4$) and fragments (3 %; $n = 1$). Of the fibers, colourless fibers were the most common colour (38 %; $n = 9$), followed by blue (25 %; $n = 6$) and red (17 %; $n = 4$). For comparison, the average amount of microplastics (before standardizing for clam mass) was 11.3 ± 6.6 particles/cultured clam and 8.4 ± 8.5 particles/wild clam (Table 1).

Discussion

There were two main objectives to this study: (1) to determine if microplastics were present in Manila clams from Baynes Sound, BC, and (2) to investigate differences in microplastic concentrations between wild and cultured clams. This study has documented microplastic presence in Manila clams from the Northeast Pacific Ocean that are consumed by humans. While concentrations in individual clams ranged from 0.07 to 5.5 particles/g, every clam had at least one microplastic item found within. This may be due to contamination in some cases, but overall these data suggest that microplastics have become ubiquitous and can enter the food chain at the benthic bivalve level.

Microplastic presence in the Northeast Pacific Ocean has been relatively understudied, despite concern from residents. Desforges et al. (2014) have documented high fiber content (~ 3000 particles/ m^3) in waters surrounding Vancouver Island, particularly in nearshore regions of the Strait of Georgia where Baynes Sound is located. Additionally, it is now clear that microplastics can enter the marine food web via zooplankton ingestion or entanglement (Desforges et al. 2015), but until now other routes of entry to food webs were unknown. In Baynes Sound specifically, Cluzard et al. (2015) documented concentrations of 0.045 microplastic particles/g (w/w) of sediment. Bivalves in the Northeast Pacific Ocean may be ingesting microplastics originating from both the water column and sediments, but detailed water sampling in Baynes Sound would provide insight into relative concentrations between water and sediment.

Microplastics have been documented in a variety of other bivalves, but mussels have been the predominant study organism to date. It is important to consider microplastic presence in other species due to economic and biological differences. Clams and mussels have very different filtration rates (Cusson et al. 2005; Hadley and Whetstone 2007), and mussels are not as economically important on the west coast of Canada as they are on the east coast (Fisheries and Oceans Canada 2015). Average concentrations in mussels (*Mytilus* sp.) range from 0.13 ± 0.14 particles/g (Vandermeersch et al. 2015) and 0.36 ± 0.07 particles/g (Van Cauwenberghe and Janssen 2014) in Europe to $\sim 2 \pm 1$ particles/g (Li et al. 2015) in China. In China, nine species of commercial bivalves were studied for microplastic contamination, including the Manila clam (*Ruditapes philippinarum*). Ark clams (*Scapharca subcrenata*) had the highest concentration of microplastics (~ 11 particles/g), followed by oysters (*Alectryonella plicatula*, ~ 7 particles/g). Manila clams had ~ 3 particles/g (Li et al. 2015), which falls in the range of values from our study.

The second objective of our study was to determine if a significant difference exists between cultured and wild Manila clams. It was hypothesized that cultured clams would have more microplastics due to proximity to plastic farm infrastructure, such as anti-predator netting, oyster culture ropes, etc. Although (on average) there were more microplastics documented in farmed clams compared with wild ones, we did not find a significant difference in microplastic concentrations between wild and cultured clams from Baynes Sound. This may be due to small sample sizes, because there was considerable variation in concentrations between sites. Furthermore, our “wild” clams were still likely influenced by the development in Baynes Sound. Selecting more remote sites away from anthropogenic development may show more significant

differences in microplastic concentrations. However, it is possible that microplastic concentrations are influenced by other factors, such as large-scale oceanographic currents and other sources of anthropogenic disturbance (e.g., wastewater, boat traffic).

Although microplastic loads in cultured bivalves have been relatively well-documented (Van Cauwenberghe and Janssen 2014; Li et al. 2015; Vandermeersch et al. 2015), comparisons between cultured and wild bivalves consumed by humans are lacking in the literature. Mathalon and Hill (2014) compared cultured and wild blue mussels (*M. edulis*) on the east coast of Canada and found farmed mussels to have significantly higher concentrations of microplastics (178 particles/mussel) compared with wild mussels (106–126 particles/mussel). In contrast, De Witte et al. (2014) conducted a quality assessment of the blue mussel (*M. edulis*) and also found farmed mussels to have mean concentrations of microplastic fibers (0.35 fibers/g) that fell between values for wild mussels from two locations along the Belgian coast (0.26 fibers/g and 0.51 fibers/g). In contrast, our study’s farmed Manila clams had 1.7 ± 1.2 particles/g (or ~ 12 particles/clam), and wild clams had 0.9 ± 0.9 particles/g (or ~ 9 particles/clam), which falls within values reported by De Witte et al. (2014) and Mathalon and Hill (2014). Differences in lab processing methods, study location, and the biology of clams versus mussels may explain the differences between these studies and our study. Specifically, mussel farming methods differ from those used to grow clams. Most east coast mussels are grown by long-line (Fisheries and Oceans 2015). The fraying of plastic-based ropes in close contact with growing mussels may influence the amount of microplastics ingested compared with other methods with fewer plastic structures (e.g., bottom or rack culture).

Qualitative features such as microplastic type and colour also can provide insight into potential plastic sources. Overall, fibers were the dominant microplastic type in our study, accounting for 90 % of the microplastics documented. Desforges et al. (2014) have documented a similar proportion of fibers (~ 80 %) in waters surrounding Vancouver Island, particularly in nearshore regions, most of which were blue, red, black, and purple fibers. The most commonly observed fibers in our study were colourless (36 %), followed by dark gray (26 %); in contrast with Desforges et al. (2014), blue, red, and purple fibers were considerably lower in abundance. Of the gray fibers recorded, 87 % were from farmed clams. It is possible the source of these dark gray fibers is the black anti-predator netting (APN) located directly above the clams, although without spectroscopic analysis (e.g., FT-IR) this cannot be verified. It has been suggested that clams might have highest concentrations of blue fibers due to the widespread use of blue polypropylene rope used on oyster farms

located near clam farms throughout Baynes Sound (Bendell 2015). In Baynes Sound, blue fibers and particles have been found in sediment samples, although other fiber and particle colours were not reported (Cluzard et al. 2015). Blue was one of the least-common fiber colours found in our study, accounting for only $\sim 6\%$ ($n = 28$) of the total observed fibers. The preliminary results of this study indicate low abundances of blue fibers are making their way into benthic organisms, but further analysis of water and sediment samples, as well as spectroscopic analysis is required.

Determining environmental microplastic contamination of field-collected organisms is difficult due to the multitude of potential contamination sources, and the subjectivity of identifying microplastic particles under the microscope. From our blank control trials, we recorded an average contamination of 5.8 ± 2.2 particles/filter paper. Farmed clams had an average concentration of 11.3 ± 6.6 particles/clams and wild clams had an average of 8.4 ± 8.5 particles/clam. If we adjust these biological samples by our contamination rate, the microplastic concentrations decrease to only approximately 5.5 particles/cultured clam and 2.6 particles/wild clam (~ 51 and 69% “proportion of contamination,” respectively). Mathalon and Hill (2014) reported contamination of ~ 100 particles/filter paper and concentrations of 178 particles/farmed mussel and 106–126 particles/wild mussel (~ 56 and 79 – 94% “proportion of contamination,” respectively). Therefore, our “proportion of contamination” is within the range of contamination reported in previous studies (although mussels and clams have differing physiology). Another important source of error lies in the use of microscopic analysis of microplastics. Song et al. (2015) reported that microscopic analysis underestimates abundances considerably compared with spectroscopic techniques (e.g., Fourier-Transform Infrared Spectroscopy [FT-IR]). As well, microscopic analysis underestimates fragments but overestimates fibers (Song et al. 2015), which could account for the large proportion of fibers noted in this study and others (Desforges et al. 2014; Mathalon and Hill 2014).

Due to the importance of the global shellfish farming industry, future work should incorporate all economically and environmentally important bivalves (clams, mussels, and oysters), as differences in bivalve physiology may affect the degree of microplastic consumption and retention. Bivalve depuration studies should be undertaken to determine if there are options to mitigate human exposure to microplastics through the consumption of shellfish. A rapidly expanding portion of the microplastics literature includes the physiological effect of microplastics in field organisms; further studies determining thresholds of tolerable microplastic concentrations and measureable

genomic and physiological responses should be a priority to help address this pollutant.

Acknowledgments Funding for this project was provided by the Natural Sciences and Engineering Research Council (Undergraduate Student Research Award), the Canada Foundation for Innovation, and the British Columbia Knowledge Development Fund. Thank you to Dr. Eric Demers and Dr. Jane Watson for reviewing early stages of this manuscript, Dr. Peter Ross and Dr. Moira Galbraith for their extensive assistance and training, and Brenna Collicutt and Robert Bourdon for development of field and lab protocols. The authors would specifically like to thank the three shellfish farmers who allowed us access to their farms and clams.

References

- Andrady AL (2011) Microplastics in the marine environment. *Mar Pollut Bull* 62:1596–1605. doi:10.1016/j.marpollbul.2011.05.030
- Arthur C, Baker J, Bamford H (eds) (2009) Proceedings of the international research workshop on the occurrence, effects and fate of microplastic marine debris. Sept 9–11, 2008. NOAA Technical Memorandum NOS-OR&R-30
- Avio CG, Gorbi S, Milan M, Benedetti M, Fattorini D, d’Errico G, Pauleto M, Bargelloni L, Regoli F (2015) Pollutants bioavailability and toxicological risk from microplastics to marine mussels. *Environ Pollut* 198:211–222. doi:10.1016/j.envpol.2014.12.021
- Bendell LI (2015) Favored use of anti-predator netting (APN) applied for the farming of clams leads to little benefits to industry while increasing nearshore impacts and plastics pollution. *Mar Pollut Bull* 91:22–28. doi:10.1016/j.marpollbul.2014.12.043
- Besseling E, Foekema EM, Van Franeker JA, Leopold MF, Kühn S, Bravo Rebolledo EL, Hefse Mielke L, Ijzer J, Kamminga P, Koelmans AA (2015) Microplastic in a macro filter feeder: Humpback whale *Megaptera novaeangliae*. *Mar Pollut Bull* 95:248–252. doi:10.1016/j.marpollbul.2015.04.007
- Boerger CM, Lattin GL, Moore SL, Moore CJ (2010) Plastic ingestion by planktivorous fishes in the North Pacific Central Gyre. *Mar Pollut Bull* 60:2275–2278. doi:10.1016/j.marpollbul.2010.08.007
- Browne MA, Dissanayake A, Galloway TS, Lowe DM, Thompson RC (2008) Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ Sci Technol* 42:5026–5031. doi:10.1021/es800249a
- Browne MA, Crump P, Niven SJ, Teuten E, Tonkin A, Galloway T, Thompson R (2011) Accumulation of microplastic on shorelines worldwide: sources and sinks. *Environ Sci Technol* 45:9175–9179. doi:10.1021/es201811s
- Claessens M, Van Cauwenberghe L, Vandegehuchte MB, Janssen CR (2013) New techniques for the detection of microplastics in sediments and field collected organisms. *Mar Pollut Bull* 70:227–233
- Cluzard M, Kazmiruk TN, Kazmiruk VD, Bendell LI (2015) Intertidal concentrations of microplastics and their influence on ammonium cycling as related to the shellfish industry. *Arch Environ Contam Toxicol*. doi:10.1007/s00244-015-0156-5
- Cole M, Lindeque P, Halsband C, Galloway TS (2011) Microplastics as contaminants in the marine environment: a review. *Mar Pollut Bull* 62:2588–2597. doi:10.1016/j.marpollbul.2011.09.025
- Cole M, Lindeque P, Fileman E, Halsband C, Galloway TS (2015) The impact of polystyrene microplastics on feeding, function and fecundity in the marine copepod *Calanus helgolandicus*. *Environ Sci Technol* 49:1130–1137. doi:10.1021/es504525u

- Comox Valley Regional District (CVRD) (2015) Regional sewer initiatives: south region. <http://www.comoxvalleyrd.ca/EN/main/departments/sewer-services/regional-sewer-initiatives/south-region.html>. Accessed 19 Feb 2015
- Cusson M, Tremblay R, Daigle G, Roussy M (2005) Modeling the depuration potential of blue mussels (*Mytilus* spp.) in response to thermal shock. *Aquaculture* 250:183–193. doi:10.1016/j.aquaculture.2005.03.045
- De Witte B, Devriese L, Bekaert K, Hoffman S, Vandermeersch G, Cooreman K, Robbens J (2014) Quality assessment of the blue mussel (*Mytilus edulis*): comparison between commercial and wild types. *Mar Pollut Bull* 85:146–155. doi:10.1016/j.marpolbul.2014.06.006
- Desforges JPW, Galbraith M, Dangerfield N, Ross PS (2014) Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Mar Pollut Bull* 79:94–99. doi:10.1016/j.marpolbul.2013.12.035
- Desforges JP, Galbraith M, Ross PS (2015) Ingestion of microplastics by zooplankton in the Northeast Pacific Ocean. *Arch Environ Contam Toxicol* 69:320–330. doi:10.1007/s00244-015-0172-5
- Duis K, Coors A (2016) Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. *Environ Sci Eur* 28:2. doi:10.1186/s12302-015-0069-y
- Eerkes-Medrano D, Thompson RC, Aldridge DC (2015) Microplastics in freshwater systems: a review of the emerging threats, identification of knowledge gaps and prioritisation of research needs. *Water Res* 75:63–82. doi:10.1016/j.watres.2015.02.012
- Endo S, Takizawa R, Okuda K, Takada H, Chiba K, Kanehiro H, Ogi H, Yamashita R, Date T (2005) Concentration of polychlorinated biphenyls (PCBs) in beached resin pellets: variability among individual particles and regional differences. *Mar Pollut Bull* 50:1103–1114. doi:10.1016/j.marpolbul.2005.04.030
- Eriksen M, Mason S, Wilson S, Box C, Zellers A, Edwards W, Farley H, Amato S (2013) Microplastic pollution in the surface waters of the Laurentian Great Lakes. *Mar Pollut Bull* 77:177–182. doi:10.1016/j.marpolbul.2013.10.007
- Fendall LS, Sewell MA (2009) Contributing to marine pollution by washing your face: microplastics in facial cleansers. *Mar Pollut Bull* 58:1225–1228. doi:10.1016/j.marpolbul.2009.04.025
- Fisheries and Oceans Canada (2013) Canadian aquaculture production statistics. Aquaculture production quantities and values. <http://www.dfo-mpo.gc.ca/stats/aqua/aqua13-eng.htm>. Accessed 19 Feb 2015
- Fisheries and Oceans Canada (2014) Species farmed in Canada. <http://www.dfo-mpo.gc.ca/aquaculture/secteur-secteur/species-especes/index-eng.htm>. Accessed 19 Feb 2015
- Fisheries and Oceans Canada (2015) Farmed mussels. Aquaculture species farmed in Canada. <http://www.dfo-mpo.gc.ca/aquaculture/secteur-secteur/species-especes/mussels-moules-eng.htm>. Accessed 19 Feb 2015
- Gillespie GE, Kronlund AR (1999) A manual for intertidal clam surveys. Canadian Technical Report of Fisheries and Aquatic Sciences 2270. Fisheries and Oceans Canada Science. Government of Canada
- Hadley NH, Whetsone JM (2007) Hard clam hatchery and nursery production. SRAC Publication No. 4301. Southern Regional Aquaculture Center, United States of America
- Hidalgo-Ruz V, Gutow L, Thompson RC, Thiel M (2012) Microplastics in the marine environment: a review of the methods used for identification and quantification. *Environ Sci Technol* 46:3060–3075. doi:10.1021/es2031505
- Hinojosa IA, Thiel M (2009) Floating marine debris in fjords, gulfs and channels of southern Chile. *Mar Pollut Bull* 58:341–350. doi:10.1016/j.marpolbul.2008.10.020
- Jamieson GS, Chew L, Gillespie G, Robinson A, Bendell-Young L, Heath W, Bravender B, Tompkins A, Nishimura D, Doucette P (2001) Phase 0 Review of the environmental impacts of intertidal shellfish aquaculture in Baynes Sound. Fisheries and Oceans Science Research Document 2001/125. Government of Canada
- Lechner A, Keckeis H, Lumesberger-Loisl F, Zens B, Krusch R, Tritthart M, Glas M, Schludermann E (2014) The Danube so colourful: a potpourri of plastic litter outnumbers fish larvae in Europe's second largest river. *Environ Pollut* 188:177–181. doi:10.1016/j.envpol.2014.02.006
- Li J, Yang D, Li L, Jabeen K, Shi H (2015) Microplastics in commercial bivalves from China. *Environ Pollut* 207:190–195. doi:10.1016/j.envpol.2015.09.018
- Lusher AL, McHugh M, Thompson RC (2013) Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. *Mar Pollut Bull* 67:94–99. doi:10.1016/j.marpolbul.2012.11.028
- Mathalon A, Hill P (2014) Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Mar Pollut Bull* 81:69–79. doi:10.1016/j.marpolbul.2014.02.018
- Mato Y, Isobe T, Takada H, Kanehiro H, Ohtake C, Kaminuma T (2001) Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environ Sci Technol* 35:318
- McCormick AR (2015) Anthropogenic litter and microplastic in urban streams: abundance, source, and fate. Dissertation, Loyola University
- Moore, CJ (2008) Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environ Res* 108:131–139. doi:10.1016/j.envres.2008.07.025
- Newton P, Gilchris A (2010) Technical summary of intrinsic vulnerability mapping methods for Vancouver Island. Vancouver Island Water Resources Vulnerability Mapping Project - Phase 2. Vancouver Island University
- Nobre CR, Santana MFM, Maluf A, Cortez FS, Cesar A, Pereira CDS, Turra A (2015) Assessment of microplastic toxicity to embryonic development of the sea urchin *Lytechinus variegatus* (Echinodermata: Echinoidea). *Mar Pollut Bull* 92:99–104. doi:10.1016/j.marpolbul.2014.12.050
- Ogata Y, Takada H, Mizukawa K et al (2009) International pellet watch: global monitoring of persistent organic pollutants (POPs) in coastal waters. 1. Initial phase data on PCBs, DDTs, and HCHs. *Mar Pollut Bull* 58:1437–1446. doi:10.1016/j.marpolbul.2009.06.014
- Rios LM, Moore C, Jones PR (2007) Persistent organic pollutants carried by synthetic polymers in the ocean environment. *Mar Pollut Bull* 54:1230–1237. doi:10.1016/j.marpolbul.2007.03.022
- Rocha-Santos T, Duarte AC (2015) A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. *TrAC Trends Anal Chem* 65:47–53. doi:10.1016/j.trac.2014.10.011
- Song YK, Hong SH, Jang M, Han GM, Rani M, Lee J, Shim WJ (2015) A comparison of microscopic and spectroscopic identification methods for analysis of microplastics in environmental samples. *Mar Pollut Bull* 93:202–209. doi:10.1016/j.marpolbul.2015.01.015
- Stolte A, Forster S, Gerdts G, Schubert H (2015) Microplastic concentrations in beach sediments along the German Baltic coast. *Mar Pollut Bull* 99:216–229. doi:10.1016/j.marpolbul.2015.07.022
- Teuten EL, Rowland SJ, Galloway TS, Thompson RC (2007) Potential for plastics to transport hydrophobic contaminants. *Environ Sci Technol* 41:7759–7764. doi:10.1021/es071737s
- Teuten EL, Saquing JM, Knappe DRU et al (2009) Transport and release of chemicals from plastics to the environment and to

- wildlife. *Philos Trans R Soc Lond B Biol Sci* 364:2027–2045. doi:[10.1098/rstb.2008.0284](https://doi.org/10.1098/rstb.2008.0284)
- Van Cauwenberghe L, Janssen CR (2014) Microplastics in bivalves cultured for human consumption. *Environ Pollut* 193:65–70. doi:[10.1016/j.envpol.2014.06.010](https://doi.org/10.1016/j.envpol.2014.06.010)
- Van Cauwenberghe L, Claessens M, Vandegehuchte MB, Janssen CR (2015) Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. *Environ Pollut* 199:10–17. doi:[10.1016/j.envpol.2015.01.008](https://doi.org/10.1016/j.envpol.2015.01.008)
- Vandermeersch G, Van Cauwenberghe L, Janssen CR, Antonio M, Kit G, Gabriella F, Michiel KJJ, Jorge D, Karen B, Johan R, Lisa D (2015) A critical view on microplastic quantification in aquatic organisms. *Environ Res* 143:46–55. doi:[10.1016/j.envres.2015.07.016](https://doi.org/10.1016/j.envres.2015.07.016)
- von Moos N, Burkhardt-Holm P, Koehler A (2012) Uptake and effects of microplastics on cells and tissue of the Blue mussel *Mytilus edulis* L. after an experimental exposure. *Environ Sci Technol* 46:327–335. doi:[10.1021/es302332w](https://doi.org/10.1021/es302332w)
- Wright SL, Rowe D, Thompson RC, Galloway TS (2013a) Microplastic ingestion decreases energy reserves in marine worms. *Curr Biol* 23:R1031–R1033. doi:[10.1016/j.cub.2013.10.068](https://doi.org/10.1016/j.cub.2013.10.068)
- Wright SL, Thompson RC, Galloway TS (2013b) The physical impacts of microplastics on marine organisms: a review. *Environ Pollut*. doi:[10.1016/j.envpol.2013.02.031](https://doi.org/10.1016/j.envpol.2013.02.031)
- Zubris KAV, Richards BK (2005) Synthetic fibers as an indicator of land application of sludge. *Environ Pollut* 138:201–211. doi:[10.1016/j.envpol.2005.04.013](https://doi.org/10.1016/j.envpol.2005.04.013)