A Comparison of Microplastics in Farmed and Wild Shellfish near Vancouver Island and Potential Implications for Contaminant Transfer to Humans

By

Cassandra Lee Murphy

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Supervisor: Dr. Leah Bendell
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COMMITTEE APPROVAL

The members of Cassandra Murphy's Thesis Committee certify that they have read the thesis titled *A Comparison of Microplastics in Farmed and Wild Shellfish near Vancouver Island and Potential Implications for Contaminant Transfer to Humans* and recommend that it be accepted as fulfilling the thesis requirements for the Degree of Master of Science in Environment and Management:

Dr. Leah Bendell [signature on file]

Dr. Bill Dushenko [signature on file]

Final approval and acceptance of this thesis is contingent upon submission of the final copy of the thesis to Royal Roads University. The thesis supervisor confirms to have read this thesis and recommends that it be accepted as fulfilling the thesis requirements:

Dr. Leah Bendell [signature on file]

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Abstract

This research compared numbers of microplastics in three species of farmed and wild shellfish collected near Vancouver Island, BC. Species included were blue mussel (*Mytilus edulis*), Manila clam (*Venerupis philippinarum*), and Pacific oyster (*Crassostrea gigas*). Soft tissue was chemically digested with nitric acid (68-70%) for 140 individuals. Significantly higher numbers of microplastics were observed in farmed blue mussels ($P = 0.021$) and Pacific oysters ($P = 0.011$), compared to their wild counterparts; whereas, no significant difference was observed between farmed and wild Manila clam ($P = 0.093$). Abundance of microplastics ranged from 5.6 microplastics/g to 657.5 microplastics/g, which are higher than any reported levels in the literature. White pellets were the most abundant microplastic particle (99%) recorded in all species. This research indicates microplastics are present in three commonly consumed shellfish species near Vancouver Island and presents a possible vector for contaminant transfer to humans.

Keywords: Microplastic, contaminant, shellfish, seafood, human health

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Introduction

There is growing public awareness and concern that microplastics in our oceans may be transferring contaminants to higher trophic organisms including humans (Brennecke, Duarte, Paiva, Caçador, & Canning-Clode, 2016, p. 1; Davidson & Dudas, 2016, p. 148; Nerland, Halsband, Allan, & Thomas, 2014, p. 18; Shim & Thomposon, 2015, p. 265; Van Cauwenberghe & Janssen, 2014, p. 65). Shellfish are filter feeding organisms that strain particulate matter from the water column to ingest nutrients. These particles can include microplastics: an abundant and ubiquitous form of plastic pollution that is present in marine environments. Microplastics can contain potentially harmful chemical additives (Deanin, 1975, p. 35) and can adsorb hazardous substances to their surface (Seltenrich, 2015, p. A37). The intersection of microplastic pollution, hazardous substances, and human exposure via the marine food web is relatively new and understudied. This field of research has received scientific attention due to the potential health implications for higher trophic organisms and humans that consume seafood (Seltenrich, 2015, p. A35).

Plastic in Oceans

Plastic is ubiquitous throughout the world's oceans due to high input volumes and exceptionally slow degradation rates (Nerland et al., 2014, p. 6). Global plastic production has increased dramatically from 1.9 tons in 1950, when large-scale plastic production began, to an estimated 330 million tons in 2013 (Nerland et al., 2014, p. 12, Seltenrich, 2015, p. A37). Approximately half of the plastic produced is intended for short-term, single use only (e.g., water bottles, food packaging) while one quarter is produced for intermediate life spans (e.g., vehicles, computers) (Mathalon & Hill, 2014, p. 69). Plastic is chemically inert which promotes durability

(Nerland et al., 2014, p. 13). However, durability becomes problematic as plastic waste can persist in the environment for decades to millennia (Mathalon & Hill, 2014, p. 69). This poses a serious threat to the environment as inputs continue to increase and plastic accumulates in both water and sediment. A study conducted in 2014 by the 5 Gyres Institute estimated there is approximately 269,000 tonnes of plastic floating on the ocean's surface, which is the equivalent to 5.25 trillion plastic particles (Seltenrich, 2015, p. A35). Previous research aimed at estimating the total volume of plastic in oceans has frequently used surface trawl nets to collect debris from the water's surface. Over half of plastic is negatively buoyant allowing it to sink towards the ocean floor and it is expected that current estimates of total plastic in oceans may be grossly underestimated as a result (Seltenrich, 2015, p. A37).

The dominant input of plastic in oceans comes from land-based sources including natural drainages, wastewater, stormwater runoff, and improper disposal of garbage. It is estimated that 4.8 million to 12.7 million tons of plastic enters oceans from land-based sources annually (Boucher, Morin, & Bendell, 2016, p. 1). Ocean-based sources include fishing, aquaculture, shipping, and illegal dumping (Nerland et al., 2014, p. 14; Bendell, 2015, p. 22).

The negative effects of macroscopic plastic on vertebrates has been thoroughly documented in fish, birds, whales, sea turtles, and other marine life (Brennecke et al., 2016, p. 1; Seltenrich, 2015, p. A37). There are a reported 660 marine species known to be affected by macroscopic plastic pollution, in many cases through entanglement or ingestion leading to reduced feeding and starvation (Claessens, Van Cauwenberghe, Vandegehuchte, & Janssen, 2013, p. 227).

Plastic pollution of varying sizes has been documented in all trophic levels including zooplankton (copepods, krill, larval mollusks, and larval echinoderms) and invertebrates (bivalves, polychaetas, and decapods) (Van Cauwenberghe & Janssen, 2014, p. 65). There is increasing evidence that microscopic plastic pollution is impacting a wide range of aquatic organisms and is presenting an ecological threat to marine ecosystems.

Microplastics and Their Fate in the Environment

Plastic is composed of organic polymers which are large molecules consisting of several repeating subunits. Plastic products are frequently derived from crude oil and they have an enormous range of applications, as seen in society today (Nerland et al., 2014, p. 12). Pure plastic is generally considered harmless due to large molecular size and properties of being biochemically inert and insoluble in water (Deanin, 1975, p.35; Lithner, 2011, p. 3310). The most common type of plastic is polyethylene (PE) followed by polypropylene (PP) and polystyrene (PS) (Desforges, Galbraith, Dangerfield, & Ross, 2014, p. 94; Nerland et al., 2014, p. 12).

Microplastics are commonly defined as small pieces of plastic less than one millimeter (Van Cauwenberghe, Claessens, Vandegehuchte, & Janssen, 2015, p. 10) or five millimeters (Claessens et al., 2013, p.227) in diameter, with the latter the more commonly used value. Despite their small size, microplastics represent the most abundant size fraction in oceans (Van Cauwenberghe et al., 2015, p. 10; Li, Yang, Li, Jabeen, & Shi, 2015, p. 3). Microscopic pieces of plastic were first documented in the early 1970s by Carpenter and Smith (1972); however, these particles would receive minimal scientific attention for the following three decades. In 2004, the term ‘microplastic’ was created by Thompson and colleagues during research conducted on

sediment and water from the United Kingdom (Thompson, Olsen, Mitchell, Davis, Rowland, John, McGonigle, & Russell, 2004, p. 838).

Microplastics can enter waterways and oceans either directly from primary sources or indirectly from secondary sources. Primary microplastics are manufactured to be microscopic in size and can include exfoliants in facial cleanser and toothpastes, abrasives in air blasting materials for cleaning rusty surfaces, vectors for drugs in medicine, and resin pellets which are used as feedstock in the fabrication of plastic (Nerland et al., 2014, p. 6; Seltenrich, 2015, p. A38). Primary microplastics escape water treatment systems due to their small size and this allows them to enter natural drainages and eventually oceans (Seltenrich, 2015, p. A37). Secondary sources originate from larger pieces of plastic that have broken down into smaller pieces by UV-radiation, mechanical wave action, physical abrasion against sediment, chemical reactions, and biodegradation (Seltenrich, 2015, p. A39; Mathalon & Hill, 2014, p. 70). The degradation of larger plastic items into smaller items is associated with compounding factors: first, the actual abundance of marine litter increases as one large piece breaks into several smaller pieces; second, the reduction in particle size exposes a wider range of organisms to ingestion and uptake; and third, the larger surface area to volume ratio of particles results in higher potential for leaching and desorption of harmful chemicals (e.g., cadmium, lead, phthalates) into organisms (COWI, 2013, p. 2; Shim & Thomposon, 2015, p. 265). The release of microfibers into wastewater during laundry washing is recognized as another secondary source of microplastics, contributing up to 100 particles per liter (Nerland et al., 2014, p. 6). The literature suggests that the largest source of microplastic pollution to the marine environment is from secondary sources (Nerland et al., 2014, p. 16) and the most abundant type of microplastic in

marine sediments is synthetic fibers, originating from the degradation of materials such as clothing, ropes, and packaging (De Witte, Devriese, Bekaert, Hoffman, Vandermeersch, Cooreman, & Robbens, 2014, p. 147). Microbeads are also considered widespread and abundant in sediments (Boucher et al., 2016, p. 7).

The fate of microplastics within the water column depends largely on the density of plastic relative to seawater (1.02 - 1.03 g/cm^3) (Nerland et al., 2014, p. 14). Higher density pieces such as polyvinyl chloride (PVC) will tend to sink while lower density items including polyethylene (PE) float (Table 1). Items of similar density to seawater such as polystyrene (PS) may remain suspended within the water column. Microplastic positioning within the water column or on sediment will impact which organisms are exposed to those pieces of plastic (e.g., filter feeder vs. deposit feeder). Other important factors that influence the distribution of microplastics in the marine environment include turbulence (e.g., wind, tide, current), plastic shape and size, biofouling, and proximity to urbanized areas (Desforges et al., 2014, p. 94; Nerland et al., 2014, p. 20).

Table 1. Common types of plastic and their proportion of total plastic production, plastic density relative to seawater, and buoyancy in seawater

Plastic Type	Abbreviation	% of Total Plastic Production	Density (g/cm^3)	Buoyancy (+/-)
Polyethylene	PE	30	0.93	+
Polypropylene	PP	19	0.91	+
Polyvinyl chloride	PVC	11	1.44	-
Polystyrene	PS	7	1.05	-
Polyurethanes	PUR	7	1.20	-
Polyethylene terephthalate	PET	7	1.38	-

Source: Nerland et al., 2014

Due to their small dimensions, microplastics are comparable in size to plankton and can be ingested by a wide range of organisms as a result, including zooplankton (e.g., copepods,

euphausiids, and larval echinoderms). Microplastic ingestion has been demonstrated in organisms with different feeding strategies such as filter-feeders (e.g., barnacles), deposit-feeders (e.g., lugworms), detritovores (e.g., amphipods), and predators (e.g., birds) (Davidson & Dudas, 2016, p.148; Mathalon & Hill, 2014, p. 80). Microplastic uptake is common in part because particles can resemble natural food sources such as plankton and fish eggs (Nerland et al., 2014, p. 18).

Recent research has demonstrated how microplastic ingestion can have detrimental effects on marine organisms. After two months of exposure to polystyrene pellets, oysters displayed significant decreases in sperm velocity, quantity and size of oocytes, and development of larvae which indicate a disruption to reproductive processes and reduced larval development (Sussarellu, Suquet, Thomas, Lambert, Fabioux, Pernet, LeGoic, Quillen, Mingant, Epelboin, Corporeau, Guyomarch, Robbens, Paul-Pont, Soudant, & Huvet, 2016, p. 2430). Other filter-feeding organisms including zooplankton, mussels, and sea cucumbers have been known to uptake microplastics with consequences such as reduced feeding, increased respiration, depletion of energy reserves, inflammation, and translocation of plastic into their circulatory systems (Sussarellu et al., 2016, p. 2430; Van Cauwenberghe et al., 2015, p. 15).

There is also growing concern that microplastics have the potential to transport invasive species from one location to another, such as the exotic barnacle, *Eliminius modestus*, which is native to Australia and New Zealand and has been observed on plastic debris in the north Atlantic Ocean (Barnes and Milner, 2004, p. 815). Transport of invasive species could pose a level of threat to native fauna and local biodiversity (Lithner, 2011, p. 3309).

Hazardous Substances Associated with Microplastics

Microplastics in marine environments often contain trace amounts of toxic, bioaccumulative, and persistent chemicals. These chemicals are either added directly to plastic during the manufacturing process (Deanin, 1975, p. 35) or indirectly through adsorption from environmental media (e.g., air, water, and soil) (Seltenrich, 2015, p. A37).

Chemical Additives

During plastic production, additives are often combined with plastic polymer to facilitate processing and to create desirable properties in commercial products. The chemical composition of additives varies but they are typically low molecular weight monomers (Lithner, 2011, p. 3010). There are over 300 known additives that are used in plastic production to provide properties such as colour, rigidity, flexibility, or softness, to name a few (Nerland et al., 2014, p.15). These additives include plasticizers, stabilizers, flame retardants, colourants, fillers, antioxidants, antimicrobial agents and several other categories of chemicals whose toxicities are not well understood (Deanin, 1975, p. 35). Several additives are known to be carcinogens, mutagens, and chemicals that are harmful to general and reproductive health (Lithner, 2011, p. 7). The amount of additive in plastic can vary drastically from 0.1% concentration in lubricants and stabilizers up to 50% in fillers, reinforcements, and plasticizers (Deanin, 1975, p. 35-37). Due to their low molecular weight, additives are weakly bound to the plastic polymer allowing for those monomers to be released from the plastic product into environmental media (e.g., air, water, food) during degradation (Lithner, 2011, p. 7).

Some examples of potentially hazardous additives used as stabilizers include arsenic, cadmium, and lead compounds (Nerland et al., 2014, p.34). Hazardous fillers can include

asbestos while pigments include elements such as cobalt, chromium and molybdenum. An organomercuric complex is an antimicrobial preservative that had previously been used in vaccinations and has since been phased out due to suspected toxicity to humans (Deanin, 1975, p. 38). More recently, bisphenol A (BPA) and phthalates have received considerable attention from the public and scientific community due to their endocrine-disrupting properties and potential negative health implications (Larsson, Bjorklund, Palm, Wennberg, Kaj, Lindh, Jönsson, & Berglund, 2014, p. 324). Plastic can contain 50% phthalates by weight and this additive improves properties of flexibility and softness (Deanin, 1975, p. 36).

Adsorbed Contaminants

Harmful contaminants can also be added indirectly to plastic surfaces and this has been demonstrated in numerous studies (Beckingham & Gosh, 2017, p. 151; Boucher et al., 2016, p. 1; Brennecke et al., 2016, p. 1). These contaminants preferentially adsorb to plastic surfaces in the presence of water and sediment due to the low polarity of plastic and the hydrophobic nature of many contaminants (Vandermeersch, Van Cauwenberghe, Janssen, Marques, Granby, Fait, Kottermon, Diogene, Bekaert, Robbens, & Devriese, 2015, p. 47). Plastic provides sorption sites for persistent organic pollutants (POPs) and heavy metals such as lead, copper, and zinc (Boucher et al., 2016, p. 7-8; Brennecke et al., 2016, p. 1; Seltenrich, 2015, p. A37). Several harmful POPs that have been detected on microplastics in the marine environment include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), and the insecticide dichlorodiphenyltrichloroethane (DDT) (Brennecke et al., 2016, p. 1; Seltenrich, 2015, p. A37). Polycyclic aromatic hydrocarbons enter the marine environment through gasoline and oil spills, effluent, and shipping activities. In contrast, PCBs

have been banned since the 1980s. However, PCBs still enter the atmosphere and hydrosphere through waste disposal, chemical processing, and agitation of sediments (De Witte et al., 2014, p. 147).

Microplastics containing harmful contaminants within or adsorbed to their surface could provide a direct route into the marine food web through ingestion.

Shellfish as a Vector for Contaminant Transfer to Higher Trophic Organisms

Microplastics can pose a direct threat to aquatic organisms through physical damage during ingestion and absorption of contaminants in the digestive tract from leaching of chemical additives and desorption of harmful substances. Nerland et al. (2014) explain that, "bivalves such as blue mussels…can filter as much as around two liters of seawater every hour and therefore it is not surprising that they have been found to contain microplastics" (p. 23). Mussels are sessile benthic organisms and this, combined with the large amount of water they process, exposes them to all harmful contaminants and pollutants within the water column. This can result in accumulation of contaminants and pollutants in their systems (De Witte et al., 2014, p. 146). Bivalves have often been used as environmental indicators in research by providing information as to the state and health of their environmental surroundings. Humans can be indirectly exposed to microplastics and associated harmful contaminants by consuming contaminated seafood, making microplastics a potential risk to human health (Vandermeersch et al., 2015, p. 46). Van Cauwenberghe & Janssen (2014) estimated that the annual dietary exposure for shellfish consumers in Europe can equate to 11,000 pieces of microplastics per year (p. 65). However, the potential risk of chemical exposure from microplastics to marine organisms and humans is not yet determined (Van Cauwenberghe & Janssen, 2014, p. 65).

The chemical pathway for harmful substances to transfer from microplastic to organisms is complex and is influenced by many variables such as plastic type, degree of weathering, chemical type, adsorption and desorption rates, environmental media, and bioaccumulation, to name a few (Beckingham & Gosh, 2017, p. 156, Brennecke et al., 2016, p. 5-6). The most common pathway for organisms to obtain contaminants is through ingestion, inhalation, and dermal absorption (Mathalon & Hill, 2014, p. 70). Ingestion will be the primary focus in the present study because it directly relates to microplastics.

Plastic composition can affect the rate of chemical adsorption or desorption which indicates that plastic type can affect bioavailability of contaminants to aquatic organisms (Beckingham & Gosh, 2017, p. 155). Brennecke et al. (2016) suggest that PVC has a higher adsorption rate compared to polystyrene due to the compound's higher surface area and the polarity of chlorine groups making the molecule more reactive (p. 1). The age of pellets also affects the bioavailability of contaminants from microplastics. Older pellets typically have larger surface area and reactivity due to weathering and biofouling (Brennecke et al., 2016, p. 2). In a study conducted by Rochman et al. (2014), metal concentration on plastic tended to increase over time during a one-year period and metal concentration did not reach saturation (Boucher et al., 2016, p. 8). An important implication of this finding is that plastics may become more heavily contaminated with metals the longer they are present in water or sediment.

After microplastics have been consumed, chemical additives and harmful contaminants are able to leach out and desorb from plastic surfaces and enter the tissue of marine organisms (Seltenrich, 2015, p. A37). There is also potential for the reverse to occur; contaminants already present within the organism could adsorb onto the surface of ingested plastic thereby reducing

the body burden of contaminants within the organism (Beckingham & Gosh, 2017, p. 156). However, this latter scenario has not been demonstrated in natural settings. In 2008, Browne confirmed that microplastics (<10 µm) can translocate from the gut tract of blue mussels into their circulatory system and hemocytes (blood cells affecting immune systems) where they can remain for two months resulting in tissue inflammation (Claessans et al., 2013, p. 228; Seltenrich, 2015, p. A40). Sussarellu et al. (2016) describe negative impacts from PAHs associated with PS particles in mussels including, "alterations of immunological responses, neurotoxic effects, and the onset of genotoxicity" (p. 2430).

Chemical migration within plastic largely depends on the size of the migrating chemical and the permeability of the polymer matrix (Lithner, 2011, p. 9). The smaller and more volatile the migrant chemical, the faster it will migrate (Nerland et al., 2014, p. 35). The rate of migration will also increase if the polymer matrix is composed of long polymer chains with large gaps between molecules where the migrant chemical can travel. However, estimating migration rates and chemical exposure becomes more challenging for complex plastic products containing several potentially harmful substances (Lithner, 2011, p. 12).

The extent of contaminant transfer from microplastic to primary consumers and indirectly to secondary consumers is poorly understood and requires further research. Several researchers caution that there may be cause for concern to human health, but not necessarily cause for alarm (Seltenrich, 2015, p. A40). However, other researchers emphasize that certain substances can be potentially harmful at any level of exposure as some substances have a non-linear response, meaning very low doses can result in acute effects (Seltenrich, 2015, p. A41). An example of this is the endocrine system and hormone regulation which are designed to function using very small

doses of signaling molecules. Introducing any substance that can interfere with essential hormones in small amounts may result in large impacts to organisms (Seltenrich, 2015, p. A41). Both BPA and alkylphenol plastic additives are endocrine disruptors that have estrogenic effects, while phthalates impact the production of testosterone (Mathalon & Hill, 2014, p. 70). Another example of a substance with no known safe limit of exposure is lead. It is a cumulative toxin that impacts the brain and nervous system and it can cause serious and permanent damage (WHO, 2017, p. 1).

Bioavailability refers to the degree and rate at which a substance is absorbed into a living organism and is a complex area of study in environmental toxicology (Beckingham & Gosh, 2017, p. 151). Microplastics consumed by organisms can be retained within the gut, translocated within the body, or egested from the body; fate largely depends on the size of the plastic particles and the size of the organism. Beckingham & Gosh (2017) explain, “microplastics loaded with hydrophobic organic contaminants (HOCs) have been shown to transfer these contaminants to organism tissues” (p. 151). When chemicals are strongly sorbed to particles, such as microplastics, the ambient concentration in surrounding environmental media decreases and those chemicals become less bioavailable to organisms due to strong bonding. Conversely, chemicals that are weakly bound to particles are more bioavailable to organisms. Plastics are generally considered to undergo reversible sorption and the sorption sites are non-competitive (Beckingham & Gosh, 2017, p. 151). The partitioning of a chemical within two immiscible phases is referred to as the distribution coefficient (D) and is a measure of solubility. To demonstrate this, Beckingham & Gosh (2017) determined that coal has a distribution coefficient for PCB-18 that is four to seven times higher than polypropylene, and polypropylene has a

higher distribution coefficient than wood (p. 154). This suggests that PCB-18 would preferentially sorb to coal, followed by polypropylene, and then wood in water. Stronger sorption to coal would result in PCB-18 being less bioavailable to organisms due to strong particle bonding, whereas, PCB-18 associated with polypropylene would be relatively more bioavailable. Therefore, the gut fluid distribution coefficient for a harmful contaminant will be an important determinant in whether the contaminant will desorb from plastic and enter the organism's tissue.

How chemicals interact with one another and what the cumulative impacts are to human health is poorly understood. There are challenges associated with studying cumulative effects of hazardous substances in humans, but it is valuable and necessary research (Seltenrich, 2015, p. A41).

Shellfish Aquaculture

Seafood provides sustenance and livelihood for billions of people around the world and is most heavily relied upon in developing nations. With a growing population, the shellfish aquaculture industry has increased in scale and intensity to meet the increasing demand for seafood products (Allsopp, Johnston, & Santillo, 2008, p. 5). Aquaculture is defined as the farming of finfish, shellfish, or aquatic plants in freshwater or saltwater environments. It has been practiced for over 4000 years overseas and has become a major industry in western society within the past four decades (Allsopp et al., 2008, p. 5).

Aquaculture supplies nearly half (45.3%) of global seafood production and is the fastest growing form of food production in the world (FAO, 2015 b, p.2). Over the past 15 years the annual growth rate of aquaculture production has been around 5.9% (FAO, 2015 b, p. 1).

Globally, the highest aquaculture production by volume for animals is from finfish (63-68%), followed by mollusks (21%), and crustaceans (10%) (FAO, 2015 b, p. 2).

Shellfish Aquaculture in Canada

Shellfish aquaculture production in Canada has nearly doubled in monetary value over a ten-year period, from $591 million in 2003 to $962 million in 2013 (DFO, 2013). The shellfish aquaculture industry produced 36,343 tons of seafood in Canada in 2015 (DFO, 2017 a). Mussels, oysters, and clams made up the vast majority (99%) of shellfish product while scallops and other types of shellfish accounted for the remaining one percent (DFO, 2017 a). Shellfish aquaculture occurs in two primary regions of Canada: on the western Pacific coast and the eastern Atlantic coast.

Cultivated Species in Canada

Blue Mussel (*Mytilus edulis*) is the most commonly farmed shellfish species in Canada and the average production value over five years (2011-2015) was $44.7 million CAD (DFO, 2017 b). Blue mussels and American cupped oysters (*Crassostrea virginica*) are cultivated in the Atlantic and are both native to the area. On the west coast of British Columbia (BC), Pacific cupped oysters (*Crassostrea gigas*) and Manila clams (*Venerupis philippinarum*) are commonly farmed species and both are introduced (DFO, 2017 b). The majority of mussel production occurs on the Atlantic coast, while most oyster production occurs on the Pacific coast (DFO, 2017 b).

Shellfish Farming Practices

Shellfish farming involves acquiring larvae from the wild or from hatchery broodstock. Small shellfish are typically raised in one of three methods: seabed culture, tube culture, or raft culture (CAIA, 2017 a, CAIA 2017 b). Seabed culture involves spreading small oysters or calms on the seafloor in intertidal grow-out areas. Anti-predator netting is commonly used for seabed culture to prevent crabs, birds, and other animals from removing individuals (BCSGA, 2016). Tube culture consists of small oysters naturally attaching themselves to rope, or placing blue mussel larvae into mesh sleeves, and then hanging rope or sleeve structures vertically in the water column, suspended from buoys or rafts (CAIA, 2017 a). In raft culture, small oysters are placed on trays which are suspended from buoys or rafts (CAIA, 2017 b). Tube culture and raft culture are off-bottom farming methods that occur in deep subtidal environments (CAIA, 2017 b).

All species are typically harvested by hand or dredge, although dredging is less commonly practiced in Canada. Off-bottom farming is considered far more sustainable than bottom-culture practices due to minimized disturbance to intertidal ecosystems (Deal, n.d., p. 23).

Shellfish as a Sustainable Seafood Source

Shellfish are considered one of the more environmentally sustainable sources of protein and will be increasingly valuable as global population and demand for seafood rise (FAO, 2016, p. 4). Current estimates indicate global population will reach 9.7 billion by the year 2050 (FAO, 2016, p. 4) Seafood production has the potential to contribute to healthy diets and global food security. Shellfish are considered to have a low environmental impact compared to other forms

of seafood production because the animals rely on clean water, produce minimal waste, and do not require feed or chemicals to grow (Deal, n.d., p. 3). However, it is also recognized that bottom-culture practices can result in habitat loss, biodiversity loss, and other detrimental effects, especially where intensive farming occurs (Deal, n.d., p. 16). Another negative impact from shellfish farming can be plastic litter in the form of anti-predator netting (APN), buoys, ropes, and rafts (Bendell, 2015, p. 22). Conversely, shellfish production and harvesting have social and economic advantages. The production of shellfish through aquaculture provides stable employment, requires minimal training, promotes equity, and generates money in rural coastal communities and developing nations (FAO, 2015 a). When all factors are weighed, shellfish aquaculture can be viewed as a sustainable industry if best management practices are applied.

Research Context

Farmed and wild shellfish often grow in settings that expose them to different population densities, water quality, pollution, and depths within the water column. Minimal research has been conducted comparing microplastics in farmed and wild shellfish for different species.

Research Question and Objective

Is there a difference in the quantity and type of microplastics in wild shellfish compared to farmed shellfish? And what are the potential implications for contaminant transfer to higher trophic organisms?

The primary objective of this research is to compare microplastic burdens in shellfish that are commonly grown in farmed and wild settings in coastal BC. The results will provide evidence as to whether there is a difference and what the possible reasons are for those observations. The second objective is to determine the possible implications for contaminant

transfer to higher trophic organisms, including humans, based on a comprehensive review of existing literature.

Methodology

Site Locations

Shellfish were purchased and harvested live from coastal waters in five locations extending north from Quadra Island, BC, and south to Puget Sound, Washington (Figure 1). The study area was approximately 340 km long, from north to south and 150 km wide, east to west.

Figure 1. Google Earth image of farmed and wild shellfish harvest locations near Vancouver Island. Shellfish species include wild blue mussel (WBM), farmed blue mussel (FBM), wild Manila clam (WMC), farmed Manila clam (FMC), wild Pacific oyster (WPO), and farmed Pacific oyster (FPO). Imagery ©2016 DigitalGlobe.

Shellfish harvest locations are characterized by semi-protected marine environments in the Strait of Juan de Fuca, Strait of Georgia, and Puget Sound. Varying amounts of residential, commercial, and industrial activity are present at each location due to the extensive study area. Local geography, wave and tidal energy, and level of exposure also vary between sites, which may impact local microplastic pollution levels (Figure 2).

Figure 2. Wild blue mussel (WBM) harvest location near East Sooke (upper left), farmed blue mussel (FBM) harvest location in Totten Inlet (upper right), wild Pacific oyster (WPO) harvest location near Qualicum Beach (lower left), farmed Pacific oyster (FPO) harvest location on Hornby Island (lower right), and wild Manila clam (WMC) harvest location in Heriot Bay and farmed Manila clam (FMC) harvest location in Drew Harbour, on Quadra Island (bottom center).

Baynes Sound, Vancouver Island and Totten Inlet, Puget Sound are well known commercial shellfish producing regions. Shellfish grown in these two locations include the following three species which were used during this research: Pacific oysters (*Crassostrea gigas*), Manila clams (*Venerupis philippinarum*), and blue mussels (*Mytilus edulis*). These three species where chosen because they are the most commercially popular bivalves sold in Canada (DFO, 2017 b). This general area near Vancouver Island was selected as a study location because it is the largest commercial shellfish producing region in BC and all three species of shellfish (blue mussels, Pacific oysters, and Manila clams) could be sourced from this area. Bivalves were chosen as the study organism because they are common, easy to sample, have a sessile lifestyle, filter large volumes of water and they are commonly used as environmental indicators (Vandermeersch et al., 2015, p. 47).

Shellfish in the present study were grouped into six categories based on species and farmed versus wild status: wild Manila clams (WMC), farmed Manila clams (FMC), wild Pacific oysters (WPO), farmed Pacific oysters (FPO), wild blue mussels (WBM), and farmed blue mussels (FBM). Four of the six groups of shellfish were purchased from two commercial suppliers, while the remaining two (WPO and WBM) were collected by hand at low tide in remote intertidal locations at Qualicum Beach and East Sooke, Vancouver Island, BC, respectively. Purchased shellfish included all farmed species as well as wild Manila clams (Table 2).

Table 2. Summary of shellfish groups, purchased or harvested status, location of origin, UTM coordinates, and commercial supplier

Shellfish Group	Purchased vs. Harvested	Location of Origin	UTM Coordinates	Commercial Supplier
WBM	Harvested	East Sooke, BC	10 U 447422 mE, 5354264 mN	N/A
FBM	Purchased	Totten Inlet, WA	10 T 500819 mE, 5222725 mN	Fanny Bay Oyster's
WPO	Harvested	Qualicum Beach, BC	10 U 386083 mE, 5470941 mN	N/A
FPO	Purchased	Hornby Island, BC	10 U 376355 mE, 5486232 mN	Mac's Oysters
WMC	Purchased	Quadra Island, BC	10 U 343471 mE, 5551127 mN	Mac's Oysters
FMC	Purchased	Quadra Island, BC	10 U 341507 mE, 5552541 mN	Mac's Oysters

1.1.1.1 Animal care form.

Royal Roads University relies on the University of British Columbia (UBC) to perform ethical reviews for research involving animals. However, ethical review and animal care forms are no longer required by UBC for any research involving invertebrates, except for cephalopods (R. Chow, personal communication, April 22, 2016).

Field Work

Purchased farmed shellfish.

Four groups of shellfish were purchased on Vancouver Island on October 22, 2016. A total of 30 FMC, 30 WMC, and 30 FPO were purchased from Mac's Oysters, and 30 FBM were purchased from Fanny Bay Oysters. Both suppliers are located in Baynes Sound and the suppliers provided information on harvest locations and farming methods and materials, where applicable (Table 3).

Table 3. Farming method and farming materials used for purchased shellfish

Shellfish Group	Farming Method	Farming Materials
FMC	Intertidal bottom culture	None
WMC	N/A	N/A
FPO	Intertidal bottom culture	None
FBM	Suspension culture	Polypropylene mesh sock, cotton sock, rope, buoys

A representative from Mac's Oysters explained that farmed Manila clams were harvested along the intertidal zone in Heriot Bay, Quadra Island and farmed Pacific oysters were harvested from northeast Hornby Island. In both locations, no farming materials were used, such as Anti-Predator Netting (APN), ropes, cages, or rafts (S. Kew, personal communications, November 28, 2016). However, these shellfish were considered farmed because the shellfish larvae seed were planted.

Wild Manila clams were harvested from the intertidal zone in Drew Harbour, Quadra Island, approximately two kilometers southeast of the farmed Manila clam harvest location in Heriot Bay (S. Kew, personal communication, November 28, 2016).

A Fanny Bay Oyster employee from the Vancouver Island store clarified that the farmed blue mussels purchased were harvested in Puget Sound, Washington. Mussels were grown via suspension culture by Taylor Shellfish Farms located at the southern extent of Puget Sound in Totten Inlet. During the juvenile phase, blue mussels are placed in polypropylene mesh socks approximately three to five meters in length (B. Dewey, personal communication, September 15, 2017). Cotton socks are typically positioned over top of the polypropylene mesh socks and the combination is suspended vertically in the water column. The cotton sock allows time for juvenile mussels to attach to the polypropylene material without being swept away by waves and currents. After approximately one to three weeks, the cotton sock disintegrates, and the blue mussels are grown out on polypropylene material (B. Dewey, personal communication, September 15, 2017).

After purchasing the shellfish, all individuals were wrapped in elastic bands to prevent the shell from opening - a possible route for microplastic contamination (Davidson & Dudas, 2016, p. 150). All individuals were then placed in previously unused, labelled and sealed Ziploc bags which were put in a cooler on ice packs.

Harvested wild shellfish.

Wild blue mussels and wild Pacific oysters were not available for purchase in Baynes Sound on the October 22, 2016 field day. A sanitary closure was also in effect in the marine area due to heavy rainfall and storm conditions during that time. A Fisheries and Oceans Canada (DFO) License for the Harvest of Contaminated Bivalves for Scientific, Experimental, or Educational Purposes was obtained on October 18, 2016 (MCFR-0 2016). The harvest license was to ensure that shellfish could be collected within the allotted two-day fieldwork window,

regardless of sanitary closures. The limited field work window was due to budgetary constraints and work schedule. All license conditions were met during collection and a report was submitted to DFO upon completion of field work.

A total of 42 wild Pacific oysters were collected by hand at low tide from Qualicum Beach on October 22, 2016. The following day, a total of 48 wild blue mussels were collected by hand at low tide from a rocky outcrop in East Sooke. A handheld Garmin GPSMAP 62s unit was used to collect UTM coordinates at each location.

1.1.1.2 Shellfish sampling protocol.

Wild Pacific oysters appeared to be evenly spread along a gravel beach within a narrow horizontal band (3m wide) at low tide, whereas, wild blue mussels were patchily distributed on bedrock within a narrow horizontal band (2m wide) at mid to low tide. The sampling strategy used for Pacific oysters and blue mussels was derived from Fisheries and Oceans Canada's Manual for Intertidal Clam Surveys (Gillespie & Kronlund, 1999). Other studies that have collected bivalves from natural settings have randomly collected individuals without a structured sampling technique (Van Cauwenberge et al., 2015, p. 11). Given that the comparison organisms were farmed individuals and a structured sampling technique was not used during their collection it may not have been necessary for wild individuals either. However, a more structured technique was chosen for wild shellfish to avoid sampling bias. At both locations, a 20m long measuring tape was placed parallel to the water line at low tide and five quadrats (2m x 2m) were spaced evenly (every 4m) along the measuring tape. Approximately eight Pacific oysters, between 7-11 cm in length, were collected by hand from each quadrat for a total of 42 Pacific Oysters. Approximately ten blue mussels, between 5-8 cm in length, were selected from each quadrat for

a total of 48 blue mussels. Lengths of wild shellfish were chosen based on the known lengths of farmed equivalent species to ensure size and age class would be comparable. Also, quadrats for blue mussels were adjusted slightly to ensure quadrats were on blue mussel clusters, due to their patchy distribution.

Following the harvest of wild shellfish, individuals were also wrapped in elastic bands to prevent the shell from opening; also referred to as gaping (Davidson and Dudas, 2016, p. 150). Wild Pacific oysters and wild blue mussels were placed in separately labelled Ziploc bags. Shellfish were immediately placed in coolers on ice packs until laboratory work commenced.

Laboratory Analysis

Shellfish were transported in coolers on ice from Victoria to Terrace by airplane. All laboratory work was completed in Terrace at Northwest Community College (NWCC) where lab space, materials and equipment were generously offered to process shellfish, chemically digest soft tissue, and identify and enumerate microplastics under the microscope.

Shellfish Preparation and Quality Control

Sample contamination is a unique and challenging aspect to all microplastics research and rigorous steps should be taken to limit airborne contaminants during sampling, handling, processing, and analysis. To achieve this, all solutions were filtered through Phenex 0.45 μm PES syringe filters inside of an HH Hawkins Ltd. conventional fume hood to remove potential contaminants. This included water sourced from AGAT Laboratories which was distilled and deionized (DDI) and arrived in unopened containers. Nitric acid (68.0-70.0%), produced by Anachemia Canada Co. (Montreal, Quebec), was provided by NWCC. Glassware was used during lab work to avoid microplastic contamination and all pieces of glassware and implements

were rinsed three times with filtered DDI water prior to use. All laboratory work was completed in a fume hood, except for microscope analysis, and foot traffic was limited in the work area to minimize airborne microplastic contamination. Nitrile gloves and a laboratory coat made of 100% cotton were worn at all times.

Shellfish wrapped in elastic bands were rinsed with filtered DDI water prior to laboratory work to remove potential microplastic contaminants from the surface of the shell. Individual shellfish were then measured for length, weight, and depth using vernier calipers (0.05mm precision). Shellfish were weighed using an Ohaus Scout Pro portable electronic balance (200g capacity) and both shelled weight and soft tissue weight were recorded to the nearest hundredth of a gram. A metal shellfish shucker, scalpel, and forceps were used to open the shell and remove soft tissue. Upon opening the shell, soft tissue was rinsed with filtered DDI water to remove intervalve water, and all soft tissue, including adductor muscles, was removed (after Vandermeersch et al., 2015, p. 48).

Chemical Digestion using Nitric Acid

The objective of chemical digestion was to break down soft tissue while retaining and isolating microplastics and other recalcitrant materials. Methods for chemical digestion were based on a recommended protocol for highest digestion efficiency using nitric acid (68-70%) provided by Claessens et al. (2013).

Shellfish soft tissue was placed in 300mL glass beakers and covered with tinfoil to limit airborne contamination. The number of shellfish placed in each beaker varied by species and soft tissue weight. This resulted in two oysters per flask, three mussels per flask, and five clams per flask (Table 4).

Table 4. Summary of total number of individuals, number of individuals per analytical batch, and effective sample size (number of batches) for farmed and wild individuals of each species

Shellfish Species and Farmed vs. Wild Status	Total No. of Individuals	No. of Individuals per batch	Sample Size (No. of batches)
Wild Blue Mussel	30	3	10
Farmed Blue Mussel	30	3	10
Wild Manila Clam	30	5	6
Farmed Manila Clam	30	5	6
Wild Pacific Oyster	20	2	10
Farmed Pacific Oyster	20	2	10

Thirty millilitres of nitric acid were added to each beaker containing soft tissue. Nine procedural blanks were also prepared using the following digestion steps, except for the addition of shellfish soft tissue. Beakers were placed in a closed fume hood at room temperature and left for overnight destruction. The shellfish liquid was then boiled (100^{o}C) over a hot plate for two hours. Foaming commonly occurred during heating and this was considered a possible source of material loss. Foam never exceeded the height of beaker walls, but it attached to the beaker walls making it challenging to recover. Foam was managed and minimized by vigorous stirring and adding small amounts of nitric acid to the beaker walls and glass stir rod to prevent and detach foam from surfaces.

Dilution and Filtration

After two hours of boiling, warm (80^{o}C) filtered DDI water was added to the shellfish liquid up to 200mL. The diluted mixture was then immediately vacuum filtered in a ceramic Buchner funnel over gridded Whatman 1005-055 Grade 5 cellulose filter paper (diameter=5.5cm, pore size = 2.5μm). Following filtration, the liquid was discarded, and each filter paper was placed in a covered petri dish for drying and temporary storage.

Microplastic Analysis

Filters were analyzed using an Olympus CHS compound microscope (40-400x magnification) with an external light source. The microscope was calibrated, and an ocular lens was used to measure the length of each particle. Filters were scanned at 40x magnification for particle enumeration and identification. Small particles that were challenging to identify were viewed under 100x magnification for more detailed observation.

Particles were identified as microplastics based on their size (< 5mm), lack of organic or cellular structures, lack of mineral or glass-like characteristics, homogenous colour, presence of fraying and equal thickness throughout their length for fiber-like particles (Marine & Environmental Research Institute, n.d., p. 3). Microplastics were examined and categorized based on their physical characteristics. Three categories of microplastic type were used in this research: fragments, pellets and fibers. Microplastic type was determined based on shape and colour. Other non-plastic particles retained on filters included silt, sand, glass, and siliceous diatoms. The following table outlines the different categories of particles observed and the associated characteristics used during identification (Marine & Environmental Research Institute, n.d., p. 3) (Table 5).

Table 5. Particle type and associated physical characteristics used for identification purposes

Particle Type	Characteristics
Microplastic fragment	Angular to sub-rounded, homogenous colour, non-reflective, no cellular structures present
Microplastic pellet	Round, homogenous colour, non-reflective, no cellular structures present
Microplastic fiber	Elongate, relatively homogenous colour, equal width throughout its length, no cellular structures present, splitting or fraying commonly observed, flexible

Particle Type	Characteristics
Sand particles	Microcrystalline, light greyish brown, sub-rounded to sub-angular grains, crumbles when prodded
Glass	Translucent, high reflectance, conchoidal fracture, sharp angular edges, brittle
Siliceous diatoms	Organic or cellular structures present throughout

Data recorded for each filter included particle type, length (longest axis), abundance, colour, and a qualitative estimate on the amount of sediment (low, moderate, high) present. Counting individual sand grains was not considered feasible from a time perspective.

FTIR Analysis

Particle analysis was completed for a sub-sample of particles using the Agilent Cary 600 Series Fourier Transform Infrared (FTIR) Microscope at a DFO facility. The spectral signature for each particle was assessed against a database of more than 250,000 known spectral signatures to determine chemical composition. The six particles analyzed included two white pellets, one black fragment, one brown fragment, one peach fragment, and one clear/white fragment. These particles were chosen due to their relative abundance and uncertainty in composition.

Statistical Analysis

Data recorded for each filter included shell length (mm), shell width (mm), shell depth (mm), shelled weight (g), soft tissue weight (g), particle type, particle length (μm), and particle colour. Data was summarized by totalling the number of microplastics in each filter and dividing by the total weight of soft tissue to determine the number of microplastics per gram of tissue. These units were chosen to standardize for shellfish size within and among species. Data was grouped into six categories based on species and farmed or wild status. Statistical tests for

normality and equal variance were performed using the software program Minitab 18 and a confidence limit of 95% ($p = 0.05$) was chosen.

Frequency histograms were plotted for six categories of data to visually assess if data was normally distributed and an Anderson-Darling test was applied to test significance.

Levene's and Bonnet's tests were applied to determine if variances were equal between farmed and wild status for each of the three species.

Data was not normally distributed for any of the three wild species groups. Therefore, the non-parametric Mann-Whitney U test was performed in Minitab to determine if the medians of two groups were equal.

To keep data transparent and simple to interpret, results were not adjusted for procedural contamination. Instead, the number of microplastics were compared between filters for shellfish and procedural blanks to determine if microplastic presence in shellfish filters was due solely to procedural contamination or to microplastics originating in shellfish tissue.

Results

FTIR Results

Particle composition analysis results indicated that three out of six particles examined were microplastics (Table 6). Two of the three microplastic particles were nearly identical white pellets and the third microplastic particle was a black fragment. The remaining three particles, initially identified as microplastic fragments by visual observation, were later determined to be non-plastic.

The two white pellets were composed of polyethylene and polyphenylene sulphide plastic material and the black fragment was determined to be a composite plastic mineralized (rubber

with a polyvinyl component) (Table 6). The remaining three non-plastic fragments were identified as mineral fibers and/or silica-based paint. Non-plastics were excluded from data analysis.

Table 6. FTIR chemical analysis results for six particles

Particle Type	Description	FTIR Analysis Results	Confidence Grade	Plastic? (Y/N)
White pellets	opaque, uniform, foamy/fibrous texture	Polyethylene (CX 1007)	B	Y
White pellets	opaque, uniform, foamy/fibrous texture	Composite plastic: Polyphenylene sulfide, ethylene, acrylic acid copolymer	B	Y
Black fragment	opaque, sub-angular	Composite plastic mineralized: Acrylonitrile/butadiene (rubber) copolymer with polyvinyl component	B	Y
Brown fragment	semi-transparent, sub-rounded	Silica-based paint or coated man-made mineral fiber	B	N
Peach fragment	semi-transparent, sub-rounded	Mineral fiber with additive similar to adhesive Ultra-temp 516	B	N
White fragment	semi-transparent, sub-angular	Mineral fiber with additive similar to adhesive ASX (sodium silicate)	B	N

Definitions: Grade B: Good degree of confidence.
A component is one of the chemical compounds in the mixture identified by FTIR; material category.

Normal Distribution of Test Results

Microplastics followed an approximate normal distribution for all farmed species and a non-normal (skewed) distribution for wild species based visually on histogram results (Figures 3-5).

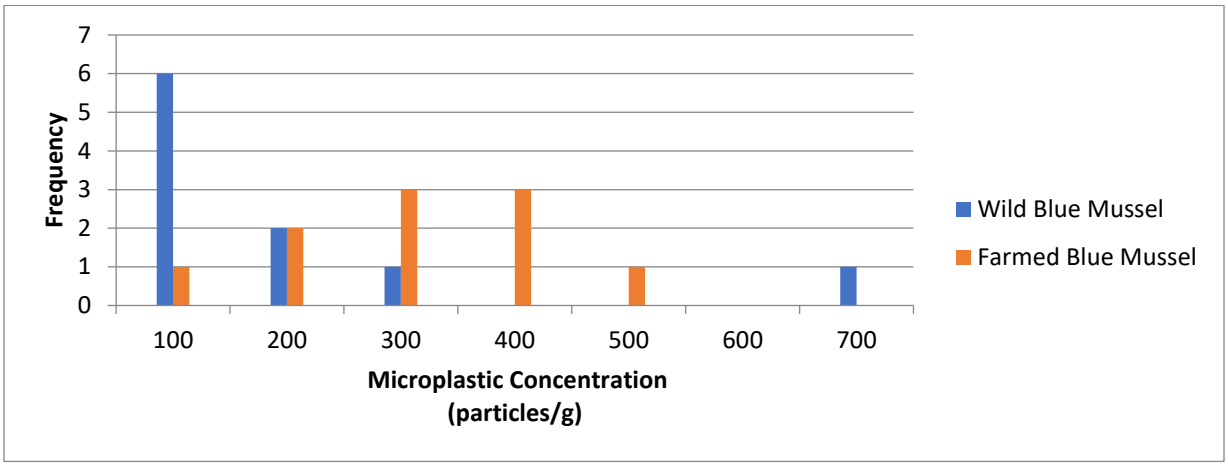

Figure 3. Frequency distribution of microplastic concentration in wild and farmed blue mussels

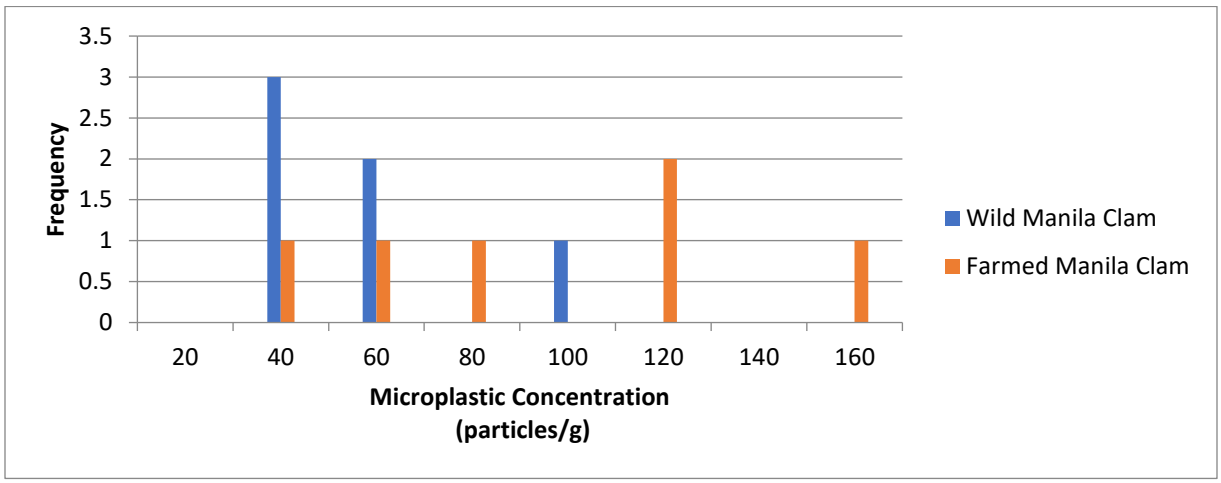

Figure 4. Frequency distribution of microplastic concentration in wild and farmed Manila clams

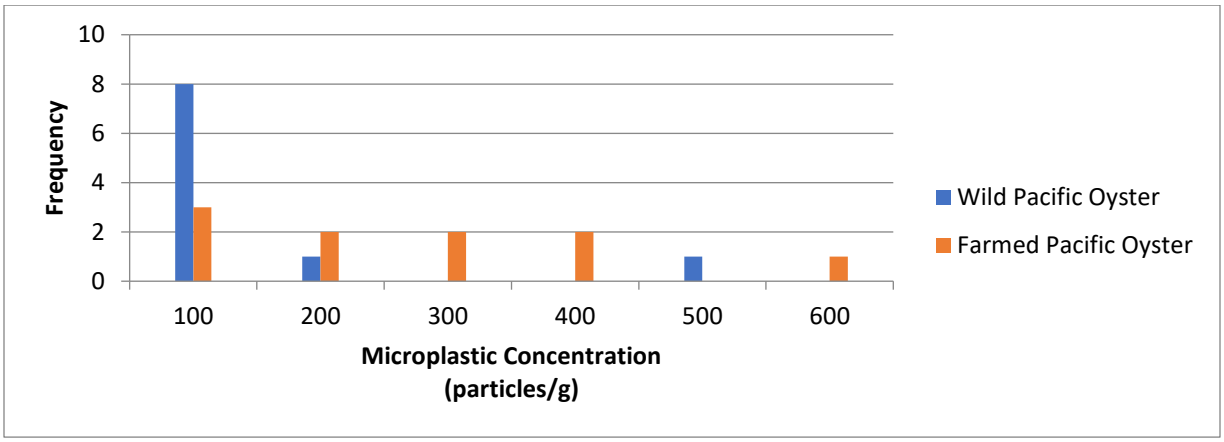

Figure 5. Frequency distribution of microplastic concentration in wild and farmed Pacific oysters

The Anderson-Darling test (Minitab) also confirm the histogram results above (Table 7). Normal data plotted along the red line and the null hypothesis was accepted ($p > 0.05$) for farmed species (Figures 6b, 6d, 6f, Table 7), and non-normally distributed data plotted off the red line and the null hypothesis was rejected ($p < 0.05$) for wild species (Figures 6a, 6c, 6e, Table 7).

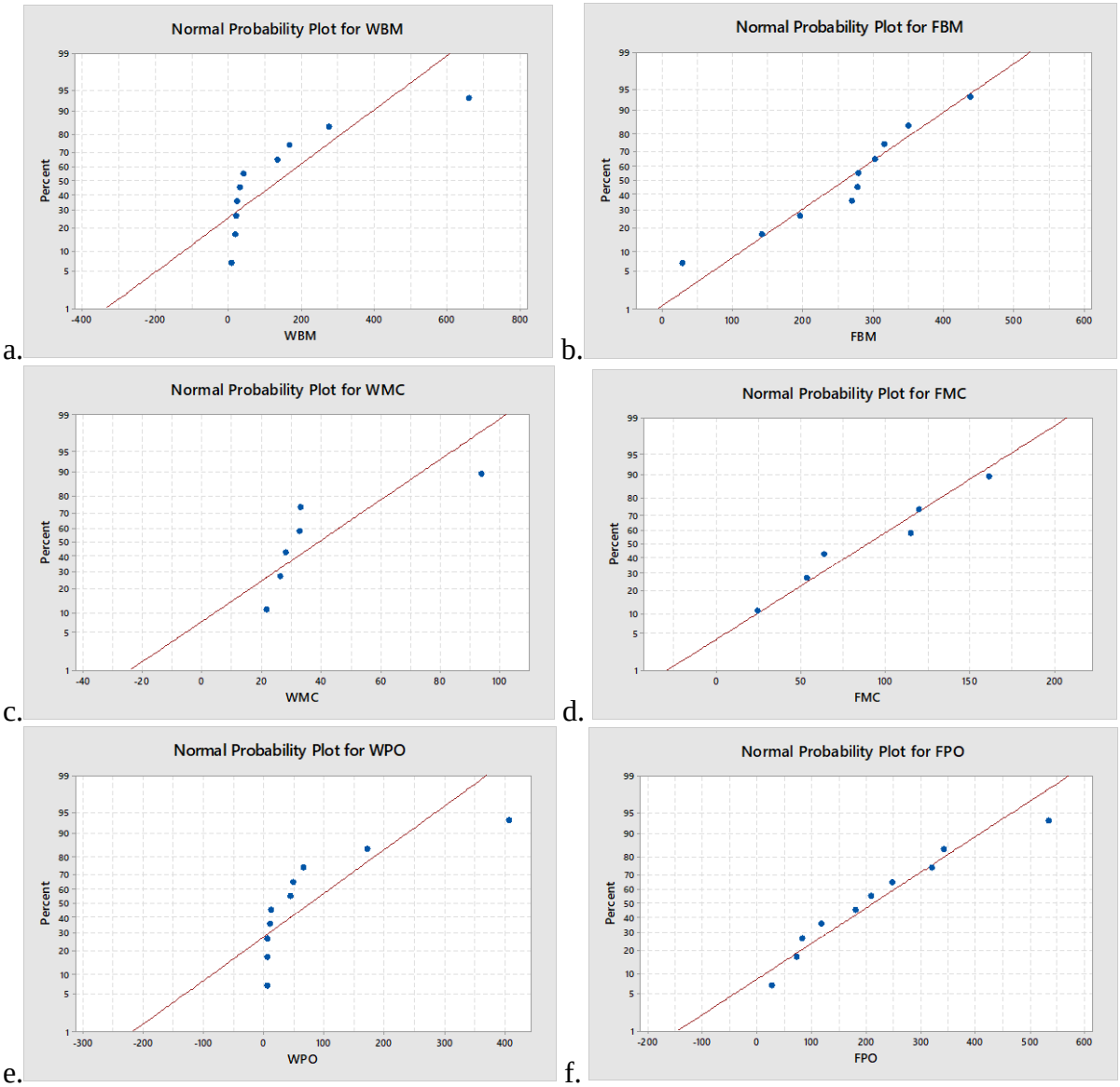

Figure 6. Normality Probability Plots generated for wild blue mussels (WBM), farmed blue mussels (FBM), wild Manila clam (WMC), farmed Manila clam (FMC), wild Pacific oyster (WPO), and farmed Pacific oyster (FPO) from the Anderson-Darling test

Table 7. Anderson-Darling statistical test results for normality for farmed and wild individuals of three shellfish species

Shellfish Species	Sample Size	Mean	Standard Deviation	AD-Value	P-Value	Null Hypothesis (Normality)
Wild Blue Mussel	10	138.00	202.30	1.29	<0.005*	Reject
Farmed Blue Mussel	10	259.40	114.10	0.34	0.409	Accept
Wild Manila Clam	6	39.20	27.08	1.07	<0.005*	Reject
Farmed Manila Clam	6	89.42	50.88	0.23	0.680	Accept
Wild Pacific Oyster	10	77.12	126.00	1.54	<0.005*	Reject
Farmed Pacific Oyster	10	212.80	153.80	0.27	0.590	Accept

Equal Variance of Test Results

Bonnet's tests revealed that all species paired by farmed and wild groups had significantly equal variances among the different shellfish groups, based on the ratio of variances ($p > 0.05$, Table 8).

Table 8. Bonnet's statistical test results for equal variance for wild and farmed pairings of three shellfish species

Shellfish Species	Standard Deviation	Variance	Estimated Ratio	Test Statistic	P-Value	Null Hypothesis (Equal Variances)
Wild Blue Mussel	202.29	40920.4	1.773	0.59	0.442	Accept
Farmed Blue Mussel	114.11	13021.8				
Wild Manila Clam	27.09	733.59	0.532	1.73	0.189	Accept
Farmed Manila Clam	50.88	2588.9				
Wild Pacific Oyster	125.99	15873.5	0.819	0.15	0.703	Accept
Farmed Pacific Oyster	153.78	23646.6				

Equality of Medians for Test Results

Since assumptions of normal distribution were not fully met in any of the species paired by farmed and wild groups examined above, the non-parametric Mann-Whitney U test was used to determine if the median number of microplastics differed significantly amongst these groups for each species.

A significant difference was found in the median number of microplastics per gram of tissue between farmed and wild individuals for both blue mussels and Pacific oysters (Table 9). In these two species, the median concentration of microplastics (particles/g) in farmed groups was significantly higher than wild groups ($p < 0.05$). There was no significant difference in the median concentration of microplastics between farmed and wild Manila clams ($p > 0.05$, see Table 9).

Table 9. Mann-Whitney U statistical test results for equal medians for wild and farmed individuals of each species

Shellfish Species	Median	Difference	W-Value	P-Value	Null Hypothesis
Wild Blue Mussels	36.61	-172.76	74.00	0.021*	Reject
Farmed Blue Mussels	277.63				
Wild Manila Clams	30.42	-36.60	28.00	0.093	Accept
Farmed Manila Clams	89.24				
Wild Pacific Oysters	27.25	-129.39	71.00	0.011*	Reject
Farmed Pacific Oysters	193.97				

*Significant result

A visual representation of mean and median microplastic concentration for farmed and wild shellfish groups can be seen in Figure 7.

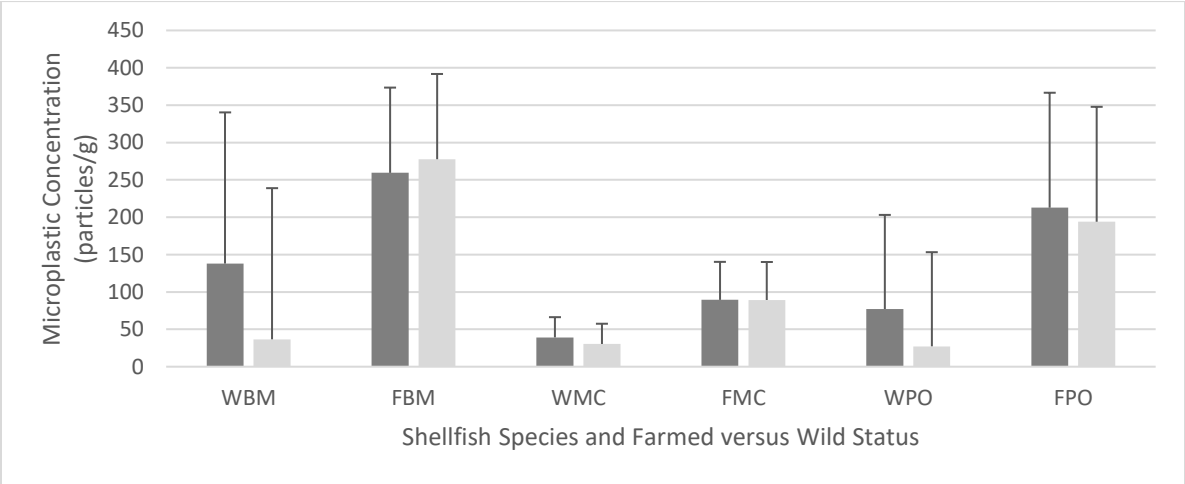

Figure 7. Microplastic concentrations (mean (dark grey) and median (light grey) particles per gram of tissue + 1 S.D.) in wild blue mussel (WBM), farmed blue mussel (FBM), wild Manila clam (WMC), farmed Manila clam (FMC), wild Pacific oyster (WPO), and farmed Pacific oyster (FPO).

A variety of microplastics, including pellets, fragments, and fibers, were observed in all 52 batches of shellfish. These results indicate that every shellfish batch, and likely each individual shellfish, contained microplastics.

Microplastic Size

The most common size class for microplastics was the smallest category, < 20 μm (53%), followed by 20-50 μm (40%), and 50-100 μm (4%) (Figure 8). All size classes greater than 100 μm, combined, accounted for approximately 3% of microplastics. In both pellets and fragments, the smallest size category accounted for more than half of the total. Conversely, fibers were more common among the middle size classes (97%), particularly between 100 μm to 2.7 mm.

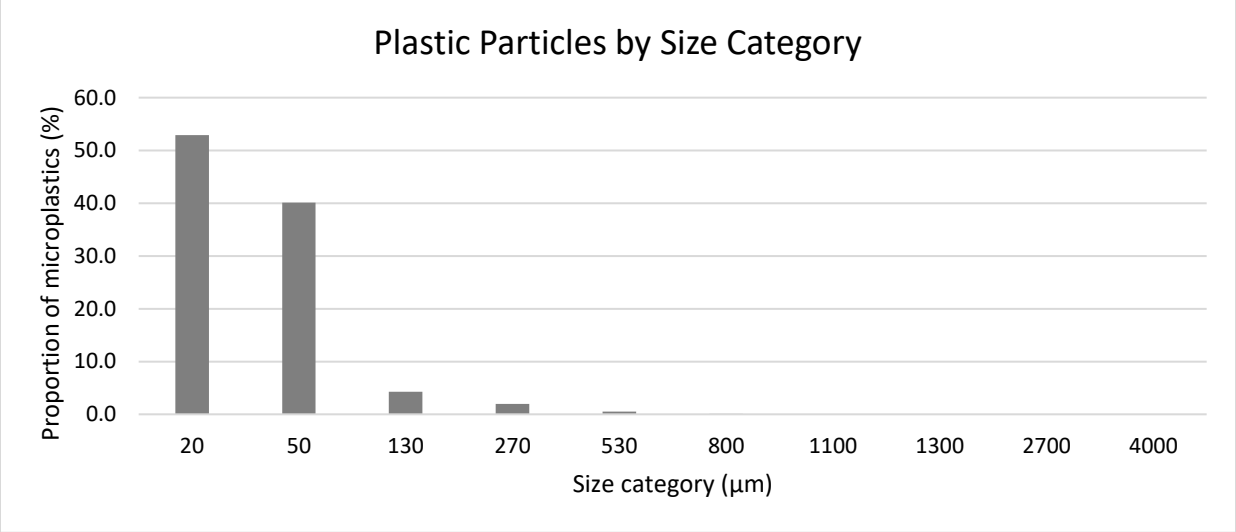

Figure 8. Proportion of microplastics (%) by size category (μm) for all filters

The largest pieces of plastic were fibers which exceeded 5mm in length in 18 fibers; these results were excluded from data analysis because they exceeded the 5mm size limit categorized as being microplastics. The smallest distinguishable pieces of plastic (limit of detection) viewed under the microscope were one half of an ocular increment wide or

approximately 10 µm in width; fibers, fragments, and pellets were all observed in this size category.

Microplastic Abundance

The lowest concentration of microplastics was observed in WPO at 5.6 microplastics/g soft tissue, while the highest concentration was observed in WBM at 657.5 microplastics/g soft tissue. It was not possible to determine how many microplastics were present in each individual because shellfish were processed in batches.

Pellets were the most common type of microplastic (98.6%) and white was the most common colour of plastic observed (Figure 9). The next most common type of microplastic was fragments (1.1%) followed by fibers (0.4%), while the most diverse colours were observed in fibers followed by fragments.

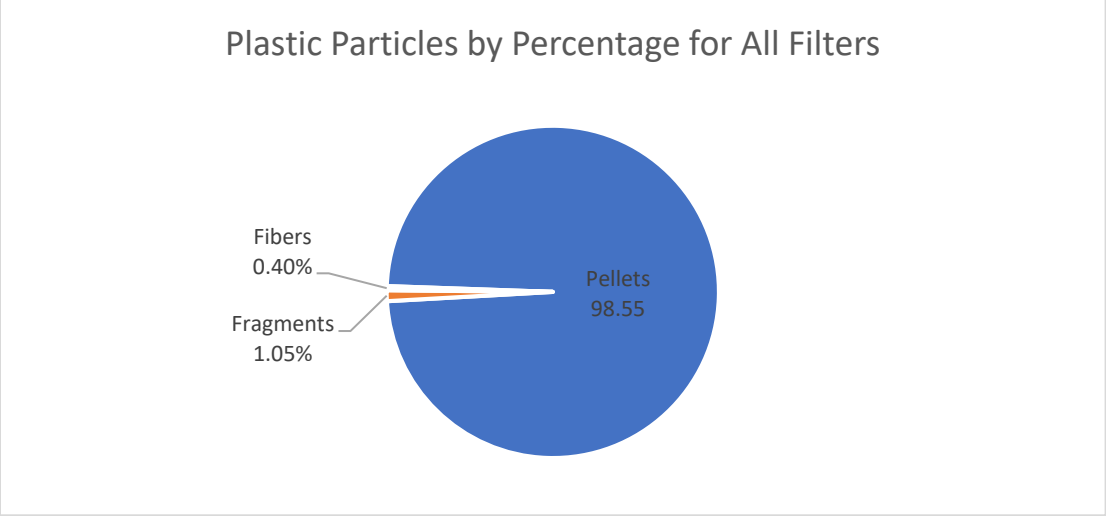

Figure 9. A comparison of plastic particle types by percentage for all shellfish filters.

Non-Plastic Particle Abundance

Of the non-plastic material identified, glass was the most common type of item followed by siliceous diatoms (Figure 10). Sand and silt grains were not enumerated due to their high

abundance and to practicality considerations. The shellfish used in the present study did not undergo a depuration period because this process is not commonly observed in the shellfish industry and humans would typically consume all materials in shellfish. Depuration would have likely resulted in fewer sediment grains, but it could have also resulted in ingested material, including microplastics, being expelled during the process (Van Cauwenberghe & Janssen, 2014, p. 66).

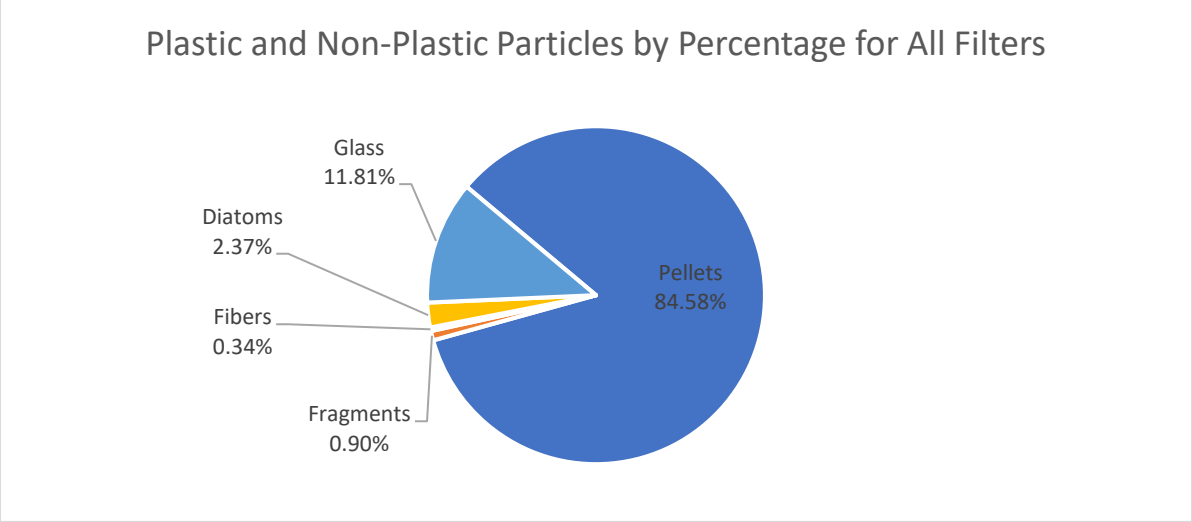

Figure 10. A comparison of plastic and non-plastic particles by percentage for all shellfish filters.

Microplastics in Wild vs Farmed Species

In blue mussels, a higher proportion of pellets was observed in farmed individuals (100%) compared to wild (92%), whereas, a higher proportion of fragments and fibers were observed in wild individuals compared to farmed (Figure 11).

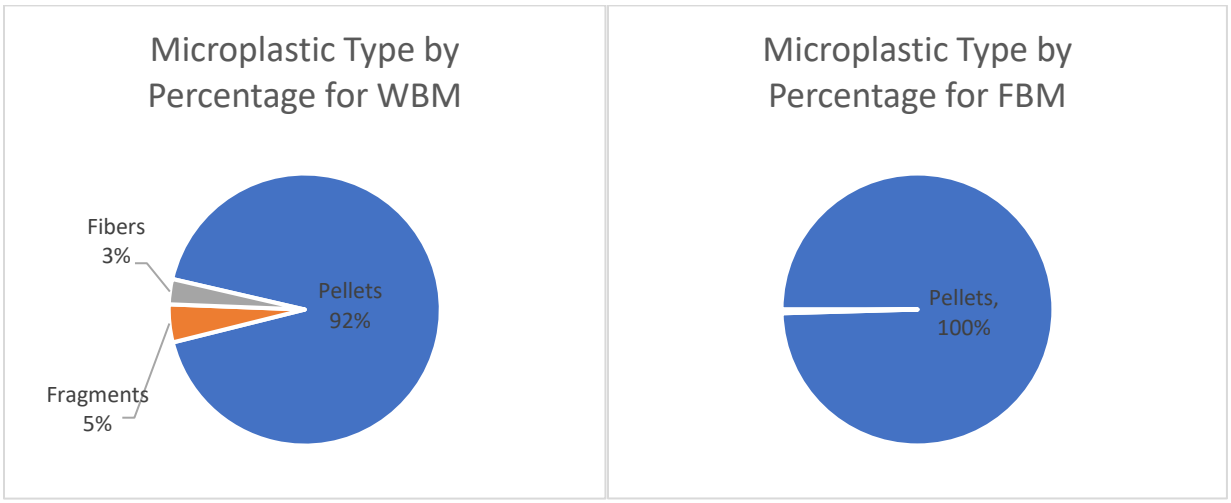

Figure 11. Proportion of microplastic type (%) in wild (left) and farmed (right) blue mussels.

In Manila clams, a higher proportion of pellet microplastics were also observed in farmed compared to wild individuals, whereas, a higher proportion of fragments and fibers were observed in wild compared to farmed individuals (Figure 12). Not included in these figures is the proportion of non-plastic particles. Glass made up a large component of particles in Manila clams. In total, WMC contained 48% glass and 52% microplastics, while farmed individuals contained 10% glass and 89% microplastics.

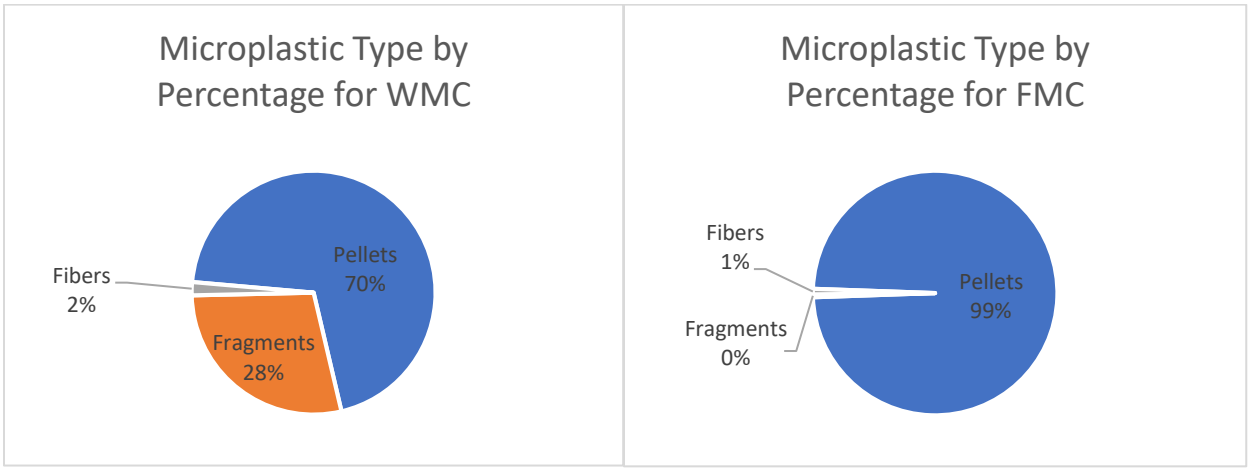

Figure 12. Proportion of microplastic type (%) in wild (left) and farmed (right) Manila clams.

Among all three species, WMC contained the highest proportion of glass (48%) followed by WPO (47%).

In Pacific oysters, the majority of microplastics in both wild and farmed individuals were white pellets (Figure 13). A higher proportion of diatoms and glass was present in WPO compared to farmed. Several small pearls (1-2 mm diameter) were also noted in wild oysters but not in farmed oysters. Pearls were extracted prior to measuring soft tissue weight and they were not included during the enumeration of particles.

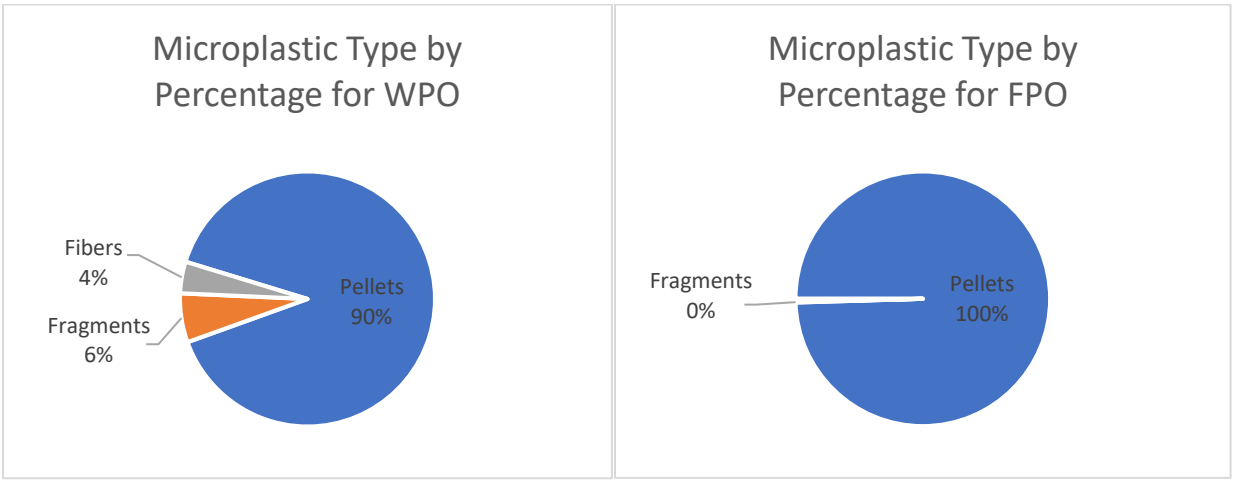

Figure 13. Proportion of microplastic type (%) in wild (left) and farmed (right) Pacific oysters.

Procedural Blanks

The average concentration of microplastic contamination in the nine procedural blank filters was 5.1 ± 4.3 (1 standard deviation (S.D.)) particles/filter (range 0-12 particles per filter). Of the nine procedural blank filters, eight contained microplastics. The most common type of microplastic observed was fibers (60%, n = 8) and the amount ranged from zero to seven per filter. The most common colour of fiber was black (57%) followed by red (18%) and grey (14%). The other type of microplastic observed on filters was fragments (40%, n = 5, range 0-6 fragments per filter). The predominant colour of fragment observed was black (63%) followed

by red (21%) and then orange (11%). No pellets were observed on any of the procedural blank filters.

The median number of microplastics in shellfish filters, for each of the six shellfish groups, was significantly higher than in procedural blank filters ($P \leq 0.002$, Table 10; Figure 14). These results confirm that microplastics observed in shellfish are not solely due to procedural contamination, with a substantial portion of the microplastics originating from shellfish tissue.

Table 10. Mann-Whitney U statistical test results for equal medians, comparing the number of microplastics in procedural blanks to six shellfish groups

Shellfish Species	Median	Difference	W-Value	P-Value	Null Hypothesis
WBM	470	464	145.00	0.000	Reject
Procedural Blanks	6				
FBM	5984	5978	145.00	0.000	Reject
Procedural Blanks	6				
WMC	626.5	620.5	75.00	0.002	Reject
Procedural Blanks	6				
FMC	2698	2692	75.00	0.002	Reject
Procedural Blanks	6				
WPO	324	318	145.00	0.000	Reject
Procedural Blanks	6				
FPO	4272	4266	145.00	0.000	Reject
Procedural Blanks	6				

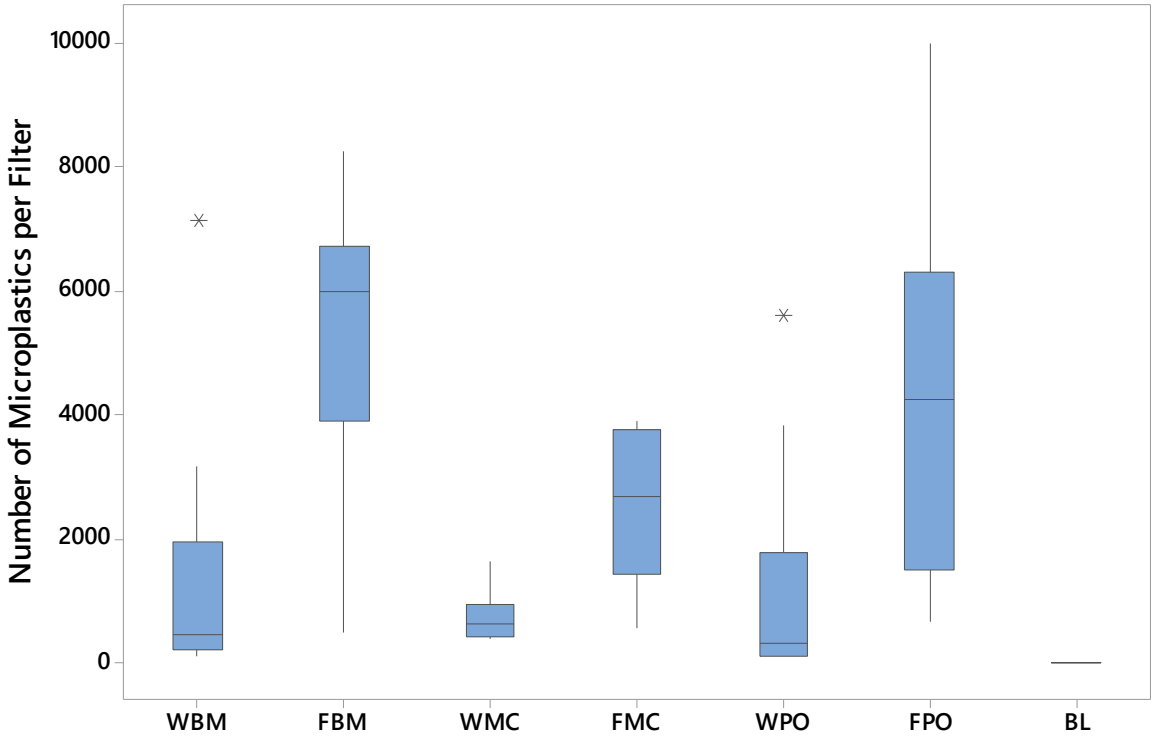

Figure 14. Boxplot diagram comparing number of microplastics per filter between wild blue mussels (WBM), farmed blue mussels (FBM), wild Manila clams (WMC), farmed Manila clams (FMC), wild Pacific oysters (WPO), farmed Pacific oysters (FPO), and procedural blanks. Note: Asterisks indicate outlier symbol.

Discussion

Comparison of Microplastic Pollution in Farmed and Wild Shellfish

Several key findings were identified from this research. Microplastics were documented in all 52 batches of shellfish, indicating widespread microplastic pollution and uptake by commonly consumed shellfish near Vancouver Island. In two of the three species investigated (blue mussels and Pacific oysters), significantly more microplastics were documented in farmed individuals compared to their wild counterparts. White micropellet was the most abundant type of microplastic (98.6%) among all species and the origin of these micropellets remains unknown. The present study documents the highest reported microplastic concentrations compared with the

literature, ranging from 5.6 microplastics/g soft tissue in WPO to 657.5 microplastics/g soft tissue in WBM. No significant difference was observed in microplastics between farmed and wild Manila clams.

One factor that may influence microplastic concentrations in shellfish is localized microplastic contaminant levels at each harvest location. This could include proximity to sources from urban centers, effluent discharge, commercial activity, and industrial activity (Mathalon & Hill, 2014, p. 77). There is an increased probability of microplastic uptake by shellfish near microplastic sources (Mathalon & Hill, 2014, p. 70).

Another factor likely to impact microplastic concentrations in shellfish is geography. Research indicates that sheltered bays provide low energy environments where particle deposition is more favourable, resulting in an accumulation of easily transported, low density microplastics in sheltered settings (Mathalon & Hill, 2014, p. 76). Conversely, exposed rocky shorelines are characterized by high energy environments where particles are more likely to remain in suspension and be flushed from the area. Boucher et al. (2016) demonstrated that higher concentrations of microplastics were present in Cates Park, a protected portion of Burrard Inlet (5560/kg wet sediment), compared to Horseshoe Bay, which is an exposed section of Burrard Inlet (3120/kg wet sediment) (p. 1).

Desforges et al. (2014) determined that microplastic abundance in surface waters around Vancouver Island appeared to be controlled, in part, by dominant oceanic processes (p. 97). Three major cities near Vancouver Island - Vancouver, Victoria, and Seattle - release their wastewater effluent into surrounding water bodies including the Strait of Georgia, Juan de Fuca Strait, and Puget Sound, respectively (Desforges et al., 2014, p. 97). Desforges et al. (2014)

measured microplastic concentrations (particles/m^3) in subsurface waters (4.5m) at several locations around Vancouver Island. This data was used to estimate microplastic concentration gradients between sample locations. The resulting gradient map displayed relatively high concentrations (6000 particles/m^3) near Quadra Island, moderate concentrations (4000 particles/m3) near Hornby Island and Qualicum Beach, and relatively low concentrations (2000 particles/m3) near Sooke (Desforges et al., 2014, p. 96).

The following table was prepared to compare variables that may be influencing microplastic concentrations at each of the six shellfish harvest locations, including level of nearby human activity, local geography/level of exposure, and estimated energy level (Table 11).

Table 11. Shellfish harvest locations, level of nearby human activity, geography/level of exposure, and estimated energy level

Shellfish Species	Location	Level of nearby human activity	Level of Exposure	Estimated Energy Level	Particle Deposition or Suspension
WBM	East Sooke, Strait of Juan de Fuca	Minor residential	Exposed, rocky shoreline	High	Suspension
FBM	Totten Inlet, south extent of Puget Sound	Moderate residential, logging, and marine commercial activity	Sheltered, mudflat	Low	Deposition
WMC	Drew Harbour, Quadra Island	Minor residential, marina, and marine provincial park	Semi-protected, gravel-sand beach	Low-moderate	Suspension
FMC	Heriot Bay, Quadra Island	Minor residential, BC ferries dock, marina, and firehall	Semi-protected, gravel-sand beach	Low-moderate	Suspension
WPO	Qualicum Beach, Strait of Georgia	Minor residential	Semi-exposed, gravel-sand beach	Moderate-high	Suspension

Shellfish Species	Location	Level of nearby human activity	Level of Exposure	Estimated Energy Level	Particle Deposition or Suspension
FPO	West Hornby Island, Strait of Georgia	Minor residential, BC ferries dock, adjacent Denman Island	Semi-protected, gravel-sand beach, <2 km wide channel	Low-moderate	Deposition

It is possible that higher microplastic concentrations were observed in FBM and FPO in the present study because those shellfish were harvested from sheltered, low energy, depositional environments where microplastics tend to accumulate. These shellfish aquaculture locations were also situated closer to urban centers where commercial and industrial activity was more prevalent. Mathalon & Hill (2014) found highest microplastic concentrations occurring along populated coastlines and within ocean gyres, as well as in areas with decreased water flow (p. 69). This theory also aligns with results for Manila clams, where no significant difference in concentration was observed. Both farmed and wild Manila clams were harvested from similar bays located less than 1 kilometer apart. The level of microplastic concentration at these two locations would likely be similar given their proximity, comparable levels of nearby human activity, and geography (see Figure 2e). Davidson & Dudas (2016) also reported no significant difference in microplastic concentration in WMC and FMC from Baynes Sound; this was attributed, in part, to small sample sizes and close proximity of the sites (p. 153). Another factor that may have impacted the results for clams versus oysters and mussels are the different feeding modes: within sediment for the former (clams) and overlying water column for the latter (oysters and mussels). Without having collected any additional data, this hypothesis cannot be tested but it is a possible explanation for higher concentrations of microplastics in farmed blue mussel and

Pacific oysters compared to wild, and no significant difference in concentration between farmed and wild Manila clams. Further study would be necessary to quantitatively determine the main drivers behind these results.

High Microplastic Concentrations in Shellfish

Based on a literature review, five other studies have compared microplastic concentrations in farmed and wild shellfish (De Witte et al., 2014; Davidson & Dudas, 2016; Li et al., 2016; Mathalon & Hill, 2014; Vandermeersch et al., 2015). Of these studies, three found no significant difference in microplastic concentration between farmed and wild shellfish, one found a significantly higher concentration of microplastics in FBM compared to WBM from Nova Scotia (Mathalon & Hill, 2014), and one found a higher concentration of microplastics in WBM compared to FBM in coastal waters of China (Li, Qu, Su, Zhang, Yang, Kolandhasamy, Li, & Shi, 2016). Four out of the five studies investigated blue mussels while the remaining study examined Manila clams (Davidson & Dudas, 2016).

Microplastic concentrations in the present study ranged from 5.6 microplastics/g in WPO to 657.5 microplastics/g in WBM, with an average of 147 microplastics/g of tissue. These appear to be the highest reported values of microplastic contamination in bivalves compared in the literature. Li et al. (2015) reported a maximum of 10.5 microplastics/g in *Scapharca subcrenata* from China (p. 2) (Table 12). In a follow up study, Li et al. (2016) documented an average 3.3 microplastics/g in mussels from heavily contaminated areas in China (p. 179). Mathalon & Hill (2014) recorded a maximum of 178 fibers/mussel in farmed blue mussels from Nova Scotia (T). However, these results cannot be easily correlated to the present study because the units are not the same, data was not adjusted for contamination, and pellets were excluded due to their close

resemblance to sediment grains (p. 73). All other studies from Europe reported less than 3 microplastics/g of shellfish tissue (Table 12), which is two orders of magnitude lower than the average concentration reported in the present study (see Figure 7). Included in these results, Van Cauwenberghe et al. (2015) report omitting all fibers from their data, highlighting the difficulty in making comparisons between studies (p. 14).

Table 12. Summary of studies determining concentration of microplastics in shellfish, chemical digest, filter paper pore size, and verification method

Author	Chemical Digest	Filter Paper Pore Size	Microplastic Concentration (microplastics/g unless stated otherwise)	Verification
Davidson & Dudas, (2016)	HNO_3	1.2 µm	WMC = 0.9 ± 0.9 FMC = 1.7 ± 1.2	None
De Witte et al. (2014)	HNO_3 $HClO_4$	10-20 µm	FBM = 0.35 fibers/g WBM = 0.38 fibers/g	Hot needle
Li et al. (2015)	H_2O_2	5 µm	Ark clam = 10.5	µFTIR
Li et al. (2016)	H_2O_2	5 µm	WBM = 2.7 FBM = 1.6	µFTIR SEM
Mathalon & Hill (2014)	H_2O_2	0.8 µm	FBM = 178 fibers/mussel WBM = 126 fibers/mussel	None
Van Cauwenberghe & Janssen (2014)	HNO_3 $HClO_4$	5 µm	FBM = 0.36 +/- 0.07 FPO = 0.47 +/- 0.16	Micro Raman Spectroscopy
Van Cauwenberghe et al. (2015)	HNO_3	5 µm	WBM = 0.2 +/- 0.3	Micro Raman Spectroscopy
Vandermeersch et al. (2015)	HNO_3 $HClO_4$	10-20 µm	FBM = 0.13 +/- 0.14 WBM = 0.18 +/- 0.14	Hot needle

The large discrepancy between the reported values in the present study and that of other authors may be attributed, in part, to different laboratory methods (e.g., chemical digest, filter paper pore size, omitting particle types, contamination mitigation techniques; Table 12). Other factors that could contribute to the discrepancy are the researcher's level of experience and the actual microplastic contamination levels in shellfish. The procedural blank filters in the present study indicate relatively low levels of procedural contamination. Therefore, procedural

contamination is likely not the cause. One interesting result of this research is that 98.6% of the microplastics identified were uniform white pellets of varying sizes (Figure 9). None of these white pellets were observed on procedural blank filters which indicates pellets likely originated from shellfish tissue.

Eight of nine procedural blank filters had relatively low levels of microplastic contamination and one had none. The average level of microplastic contamination was 5.1 ± 4.3 (1 S.D.) particles/filter (range 0-12 particles per filter). This level of contamination is relatively low compared to Mathalon and Hill (2014) who reported an average of 100 fibers per filter from airborne contamination (p. 76). In comparison, Van Cauwenberghe & Janssen (2014) reported no airborne contamination on any of their filters (p. 66). Davidson & Dudas (2016) reported an average of 5.8 ± 2.2 particles/filter (range 3-8 particles/filter) which compares to the levels of contamination reported here (p. 152; see Table 10). The mitigation measures used in the present study were similar to those used by Davidson & Dudason. In comparison, Mathalon & Hill did not describe rinsing shells or placing elastic bands around shells to prevent gaping. There was also no mention of filtering reagents prior to use, consistently covering beakers, conducting all work inside a fume hood, preferentially choosing glassware over plastic, or wearing cotton clothing only. It appears that less stringent mitigation measures have a considerable impact on procedural contamination levels.

Abundant White Pellets

The highest known concentration of microplastics in shellfish tissue, in comparison to the literature, is being reported in the present study (657.5 microplastics/g for WBM). Of these

microplastics, nearly 99% were opaque, white to dull yellow, foamy to fibrous white pellets of various sizes.

Two white pellets were selected for FTIR analysis. Results indicate the two pellets are made of plastic; specifically, polyethylene and polyphenylene sulfide/ethylene/acrylic acid copolymer (see Table 6). Polyethylene is one of the most commonly produced plastics and it is primarily used in packaging, but it also in abrasives, precursor pellets and fishing gear (e.g., nets, ropes, fishing line) (Desforges et al., 2014, p. 94 & 97; Mathalon & Hill, 2014, p. 70). Polyphenylene sulfide is an engineering plastic often used in high-performance thermoplastics in a variety of industrial applications (Meyer, Carnevale, & Bersee, 2010, p. 1).

While composition helps provide information about the origin of plastic, it does not definitively determine the source. It is unlikely that the white pellets originated solely from shellfish farming operations given that two out of three farmed shellfish (FMC and FPO) were not known to be in direct contact with plastic farming materials (e.g., nets, ropes, buoys, cages, etc.). Furthermore, one of the FTIR analysis results indicated the material type to be an engineering thermoplastic. Micropellets observed in shellfish likely originated from local input sources such as effluent, storm water runoff, bilge water, or commercial and industrial operations. However, it is worth noting that the shellfish aquaculture makes extensive use of high density polyethylene (HDPE) (Desforges et al., 2014, p. 97) and the presence of polyethylene was confirmed in the present study.

Recent microplastic research conducted near Vancouver and Vancouver Island also revealed high concentrations of microplastic pellets in sediment (Kazmiruk, Kazmiruk, & Bendell, 2017, p. 2). Kazmiruk et al. (2017) documented microplastic pollution at all 16 sample

sites in Baynes Sound and Lambert Channel, indicating widespread contamination of their study area, which overlaps with the study area in this report. Micropellet abundance was approximately 99% higher (25000/kg wet sediment) than microfibers (100-300/kg wet sediment) and microfragments (100-300/kg wet sediment) in intertidal sediment (p. 2). At three sample sites, the proportion of microplastics was high enough to be comparable to organic matter and silt content. In a different study, Boucher et al. (2016) measured up to 5560 microplastics/kg wet sediment in a protected part of Burrard Inlet (p. 1). Of these microplastics, approximately 75% were categorized as microbeads. The other possible reason for high pellet abundance compared to fibers and fragments could be the degradation of certain fiber types due to chemical digestion (HNO_3) (Claessens et al., 2013, p. 232).

Potential Contaminant Transfer from Microplastics in Seafood to Humans

Studies have documented microplastics in common seafood items including mussels, clams, and oysters (Davidson & Dudas, 2016; De Witte et al., 2014; Li et al., 2015; Li et al., 2016; Mathalon & Hill, 2014; Van Cauwenberghe & Janssen, 2014; Van Cauwenberghe et al., 2015; Vandermeersch et al., 2015). In the present study, microplastics were observed in all 52 batches of mussels, clams, and oysters indicating widespread microplastic pollution and uptake by shellfish near Vancouver Island.

Variables Impacting Contaminant Behaviour

1.1.1.3 Contaminant Mixtures and Interactions

Several variables impact the behaviour of contaminants, making this area of microplastics research particularly challenging. Previous studies have examined sorption and desorption rates

of contaminants in isolation (Bakir Rowland, & Thompson, 2012, p. 2782). Bakir and colleagues (2012) recognized the need to research organic contaminants as complex mixtures which more closely resembles the natural environment (Bakir et al., 2012). These authors studied the sorption of two contaminants (4,4'-DDT, Phe) onto plastic (PVC, PE) from a single mixture. There was no difference in the sorption capacity of DDT onto plastic in either a single-solute or a bi-solute system with Phe. However, Phe had decreased sorption capacity in the presence of DDT, suggesting DDT outcompetes Phe for sorption sites on plastic (Bakir et al., 2012, p. 2782). These results are somewhat predictable as DDT is more hydrophobic than Phe, illuminating the complex nature of contaminants and their synergistic or antagonistic effects when present as mixtures.

1.1.1.4 Plastic Composition

Plastic composition can also affect the behaviour of contaminants. Features such as density or molecular structure, shape, polarity, level of weathering, and age can impact the rates of contaminant adsorption and desorption (Bakir et al., 2012, p. 2785; Brennecke et al., 2016, p.1). The rates tend to increase with less dense polymers containing larger pores, with larger surface area, higher polarity (reactive groups), and increased age.

1.1.1.5 Chemical Speciation

Metals such as mercury (Hg) and lead (Pb) partition into various chemical forms which affect bioavailability (Clarkson, T. W., & Magos, L., 2006, p. 611; Suedel, B.C., Nicholson, A., Day, C.H., & Spicer, C., 2006, p. 355). While chemical speciation has not been shown to impact the behaviour of contaminants directly with microplastics, it does affect the contaminant's

behaviour with living organisms which can lead to health implications (Clarkson & Magos, 2006, p. 610).

Contaminant Behaviour under Simulated Physiological Conditions

Studies have estimated the rates of adsorption and desorption of contaminants from microplastics; however, these experiments are often conducted in the absence of gut surfactants which act to simulate physiological conditions. Bakir investigated contaminant (Phe, DDT) desorption rates from plastic (PVC, PE) in the presence of gut surfactants. Desorption rates were up to 30 times higher in the presence of gut surfactants compared to seawater (Bakir, Rowland, & Thompson, 2014, p .17). This aligns with other studies which have noted higher contaminant recoveries and increased desorption rates for Phe and PAHs in the presence of gut surfactants (Bakir et al., 2014, p. 17).

In addition to surfactants, pH and temperature can be adjusted to simulate warm-blooded versus cold-blooded organisms. Bakir et al. (2014) documented a further increase in desorption rates in higher temperature conditions in the presence of surfactants; this suggests warm-blooded animals may be at risk of increased exposure to contaminants (p. 16).

Relative Importance of Microplastics Compared to Other Exposure Routes

Bakir investigated the role of contaminant transfer from microplastics to organisms, compared to other exposure routes, using a holistic and systematic approach (Bakir, O'Connor, Rowland, Hendriks, & Thompson, 2016, p. 56). The authors recognized the need for a novel approach because recent modelling has tended to exclude the role of gut surfactants, gut retention time, organism type, pH, and temperature; all of which can substantially affect desorption rates. Bakir et al. (2016) modelled the transfer of three contaminants (DDT, Phe, and

bis-2-ethylhexyl phthalate (DEPH)) from microplastics (PVC, PE) to three organisms representing different feeding strategies: a benthic invertebrate, a seabird, and a fish (p. 56). Results concluded that uptake from food and water was the main exposure route for DDT, Phe, and DEHP; whereas, the relative uptake of contaminants from plastic was negligible (p. 63). In a separate study, the uptake of contaminants was estimated to be 21,000 times higher by ingestion of prey compared to microplastics (Bakir et al., 2016, p. 63). The implications of these results mean that microplastics may provide a negligible to small vector for contaminant transfer to organisms, relative to other processes such as feeding.

Although recent modelling indicates microplastics have negligible impact on contaminant uptake by organisms, when compared to ingestion and respiration, these results should be interpreted with caution. Models have predominantly considered sorbed contaminants. These models have excluded the role of additives which can be present in polymers in extremely high concentrations (up to 80% by weight) (Bakir et al., 2016, p. 63). Also worth noting are cumulative impacts to organisms from both additives and sorbed contaminants.

Typical model parameters include relatively short retention times reflective of gut conditions. This is problematic as evidence suggests that small microplastic particles can translocate from the gut tract of blue mussels into their circulatory system (Mathalon & Hill, 2014, p. 77). If particles remain in other tissues for extended periods of time this would likely result in higher contaminant release compared to the digestive tract. In addition, nanoplastic particles (< 100 nm in diameter) are relatively understudied and are more numerous than microplastic (Koelmans, A. A., Besseling, E., & Shim, W. J., 2015, 325). These particles are

smaller than microplastics, with larger surface area, and they are more likely to translocate, desorb contaminants, and reside in tissue for longer periods of time (Bakir et al., 2016, p. 63).

Finally, there are certain contaminants that can be potentially harmful at any level of exposure. Phthalates are endocrine disruptors which impact hormone regulation at very low doses (Seltenrich, 2015, p. A41). Phthalates are added to plastic to improve flexibility and softness and can account for 50% by weight (Deanin, 1975, p. 36). While microplastics appear to play a small role in contaminant transfer, it may be a critical role for contaminants with no known safe level of exposure.

Conclusion

In examining microplastic pollution in farmed and wild shellfish near Vancouver Island, significantly higher concentrations of microplastic were observed in farmed blue mussels and farmed Pacific oysters compared to their wild counterparts. No significant difference was observed between farmed and wild Manila clams. All shellfish batches contained relatively high numbers of microplastics suggesting widespread plastic pollution at harvest locations. The average level of microplastic pollution in shellfish was 147 microplastics/g of tissue, with a minimum concentration of 5.6 microplastics/g in WPO and a maximum concentration of 657.5 microplastics/g in WBM. These results appear to be the highest reported values of microplastic contamination in shellfish compared with the literature. Approximately 99% of these microplastics were white pellets composed of PE and polyphenylene sulfide/ethylene/acrylic acid copolymer. The origin of these white pellets is unknown; however, they were likely present in shellfish tissue as no white pellets were observed in procedural blank filters. It is possible that microplastic pollution observed in shellfish reflects the local levels of contamination in water

and sediment. However, additional data would need to be collected to determine the drivers behind the contaminant levels observed.

Recent modelling suggests microplastics may play a negligible to small role in contaminant uptake by organisms in comparison to other exposure routes, such as ingestion and respiration. However, these results should be interpreted with caution. Models often exclude important factors such as additives, gut surfactants, pH, and temperature and they have not accounted for the role of nanoplastics (<100 nm in diameter), which are thought to be more numerous and bioavailable than microplastics (Koelmans, A., Besseling, E., and Shim, W., 2015, p. 325). In addition, there are certain contaminants with no safe limits of exposure including lead and phthalates. As the global demand for plastic continues to rise, so too will the level of microplastic uptake by filter-feeding organisms.

The anomalously high levels of microplastics reported in the present study emphasize the need for standardized methodology to isolate and identify microplastics in shellfish. This standardized method should consider digestion efficiency, recovery of plastics, visual analysis, a secondary means of confirming chemical composition, cost, sources of error, and consistent data collection (e.g., units). The need for standardization is crucial if we are to determine baseline levels of contamination in shellfish and assess the potential risk to human health.

It remains unclear which digest option is optimal for destroying shellfish tissue and recovering different types of plastics. A study investigating common plastic types and a variety of digestion options (e.g., acids, bases, oxidizers, enzymes) is recommended to determine which digest retains the most types of plastic while efficiently breaking down tissue.

It is recommended that additional data be collected when investigating microplastic concentrations in farmed and wild shellfish, if resources permit. Data can include water and sediment samples from harvest locations, geographical data, shellfish density, and water samples from potential microplastic sources (e.g., bilge water discharge, effluent, stormwater runoff). Determining what factors have the greatest influence on microplastic pollution in shellfish could influence the selection of farm location and farming practices.

Future studies investigating the risk of contaminant transfer from microplastics in shellfish to humans, should account for variables that are not often incorporated into models. These variables include chemical additives in plastic, gut surfactants, synergistic or antagonistic effects present in complex mixtures, and the role of nanoplastics.

Minimal information is known about the distribution and abundance of nanoplastics in marine environments, the uptake and movement of nanoplastics in organisms, retention times, and overall body burdens. Nanoplastics presents a new area of research that will impact our understanding of the risk microplastics pose to human health.

Finally, studies near Vancouver Island have reported high levels of micropellets, and their source is unknown. A study examining the source of micropellet pollution in nearshore marine environments near Vancouver Island would be valuable in reducing micropellet pollution in those areas.

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APPENDIX A
Potential Sources of Error

Extensive effort went into preventing airborne microplastic contamination during harvest, purchase, digestion, filtration, and microplastic enumeration. However, contamination could have been introduced during all stages of shellfish preparation. The following sections describe quality control measures implemented to prevent and quantify contamination, reduce sample loss, and minimize human error during laboratory work.

Harvested Wild Shellfish

Although far fewer uncertainties are present for shellfish harvested directly by the researcher, as compared to purchased, potential sources of microplastic contamination exist and are listed below:

- Harvest; clothing and air contamination during harvest;
- Storage; new Ziploc bag, residual water, and air contamination during storage; and
- Stress; ingested materials may have been egested during handling or storage.

Purchased Farmed Shellfish

When purchasing shellfish, there is limited knowledge of the farming method, handling practices, timing, and exposure to other materials prior to purchase (Lusher, Welden, Sobral, & Cole, 2017, p. 1347). There may also be less information available about local environmental conditions such as water quality, effluent, and nearby residential, industrial or commercial activity.

Other complicating factors or sources of contamination include:

- Sampling technique; randomized sampling is highly unlikely and not expected;

- Handling technique; unknown if plastic materials (e.g., gloves, clothing) were used; handling duration and frequency are unknown (e.g., physical movement and stress);
- Harvest; unknown if plastic materials (e.g., tools or nets) were used;
- Storage conditions; unknown if plastic materials (e.g., bags, sacks) were used and what environmental conditions were present during storage and transport;
- Timing; unknown what length of time animals were off ice or stressed, if material was egested, and how long animals were out of water from harvest to sale; and
- Other sources of external contamination; if gaping occurred, unknown level of contamination from air, ice, or water.

Chemical Digestion and Filtration

Nitric acid (68-70%) was chosen over bases and oxidizers due to its exceptional efficiency at breaking down soft tissue under optimal conditions (Claessens et al., 2013, p. 232). There are benefits and disadvantages for each chemical option. Although nitric acid is known to preserve microplastic fragments and pellets (Davidson & Dudas, 2016, p. 151), it is also known to break down nylon fibers (Claessens et al., 2013, p. 232). Other types of synthetic fibers may be capable of resisting degradation when exposed to nitric acid, such as polyethylene (e.g., fishing trawl nets) and polypropylene (e.g., crab entangling nets, drift nets, trawl nets) (Vandermeersch et al., 2015, p. 53). A disadvantage to using a chemical other than nitric acid, such as hydrogen peroxide (H_2O_2), is incomplete destruction of soft tissue and partial melting of fragments and pellets (Vandermeersch et al., 2015, p. 51). Other more recent recommended digestion protocols include potassium hydroxide or enzymes for efficient tissue break down and recovery of microplastics (Lusher et al., 2017, p. 1355).

Despite high digestion efficiency using nitric acid, a relatively minor greasy tissue fraction precipitated when warm (80^{o}C) filtered DDI water was added to the boiling nitric acid shellfish liquid (100^{o}C). Other authors suggest using a chemical digest mixture containing both nitric acid and perchloric acid to help reduce the greasy tissue fraction (De Witte et al., 2014, p. 148, Vandermeersch et al, 2015, p. 48).

Microplastic Enumeration

Sources of error and contamination that may have impacted microplastic enumeration include the following:

- Greasy tissue fraction; residue may coat or obstruct the view of microplastics;
- Sediment; large particles may obstruct the view of microplastics;
- Low contrast; difficult to see clear or white microplastics against white filter paper;
- Human error; eye fatigue, double counting;
- Minimal experience; reliable identification requires training and experience; and
- Limit of detection; challenging to distinguish particles < 10µm in diameter.

Extensive efforts were implemented to prevent human error; however, some sources of error and contamination are challenging to control and are also difficult to quantify.

APPENDIX B
Quality Control Mitigation Measures

In any research involving microplastics it is critical to include stringent quality control measures to minimize contamination. A summary of the quality control measures used to prevent contamination, quantify contamination, reduce sample loss, and minimize human error during laboratory work, is as follows:

- Wiping down all surfaces prior to commencing lab work
- Bounding shellfish in elastic bands to prevent gaping
- Filtering DDI water and other solutions through 0.45µm syringe filter
- Rinsing shellfish to remove microplastics from shell exterior
- Using primarily glassware to prevent microplastic contamination
- Rinsing glassware and implements three times with filtered DDI water prior to use
- Covering beakers with tinfoil or filters with petri dish covers when not in use
- Vigorously stirring shellfish liquid to reduce foam
- Adding small amounts of nitric acid to beaker walls and glass stir rod to prevent and detach foam from surfaces
- Wetting down filter surface with filtered DDI water prior to pouring sample into Buchner funnel to create seal and avoid sample loss below filter
- Preparing nine procedural blanks to assess procedural contamination
- Wearing nitrile gloves and 100% cotton lab coat
- Conducting all laboratory work inside fumehood
- Limiting foot traffic near work area in classroom laboratory setting
- Gridding filter paper to create systematic approach and to reduce likelihood of double-counting