Application of the Blue Mussel (*Mytilus edulis*) as an indicator of microplastic pollution within the Salish Sea

by

Julie Dimitrijevic

B.Sc. (Biology), University of Victoria, 2011

Thesis Submitted in Partial Fulfillment of the
Requirements for the Degree of
Master of Science

in the
Department of Biological Sciences
Faculty of Science

SIMON FRASER UNIVERSITY
Fall 2018

Approval

Name:	**Julie Dimitrijevic**
Degree:	**Master of Science (Biological Sciences)**
Title:	**Application of the Blue Mussel (*Mytilus edulis*) as an indicator of microplastic pollution within the Salish Sea**
Examining Committee:	**Chair:** Gail Anderson Professor
	Leah Bendell Senior Supervisor Professor
	Peter Ross Supervisor Vice-President of Research Ocean Wise Conservation Association
	Anne Salomon Supervisor Associate Professor School of Resource & Environmental Management
	Chris Harley Supervisor Professor University of British Columbia Department of Zoology
	Vicki Marlatt Examiner Assistant Professor
Date Defended/Approved:	November 27, 2018

Abstract

Plastic polymers less than 5 mm in diameter, called microplastics (MPs), are an emerging contaminant of concern impacting marine organisms globally. The blue mussel (*Mytilus edulis*) is a prominent bioindicator used to quantify the accumulation of lipophilic contaminants to assess the health of marine environments. For this reason, blue mussels were utilized to establish baseline MP abundances in British Columbia (BC) and assess the practicality of using mussels as indicators of MP pollution. Mussels (n = ~15, 000) were placed in cages at 11 locations within the Strait of Georgia and southern BC waters in the winter of 2017. Mussels were sampled on Day 0, Day 30 and Day 60 post deployment and MP abundances quantified. For all sites combined, a total of 336 suspected microplastics (SMPs) were identified in 171 mussels, resulting in an average of 1.96 (0.13 SE) SMPs per mussel. After correcting for contamination and standardizing for weight, mean SMP abundances averaged 0.43 (0.06 SE) CSMP/GWW (gram wet weight). 91% of the SMPs enumerated were microfibers. A two-factor complete randomized design analysis of variance revealed that mean CSMP/GWW differed significantly over the 60-day period between the 11 sites ($p = 0.0003$), however, only mussels at the T60 – Powell River site had significantly more CSMPs/GWW. Furthering this, Fourier Transform Infrared Spectroscopy identified a total of 11 of 66 SMP particles (17%) as plastic. A complimentary exposure experiment was conducted in the spring of 2018 to assess particle fate post mussel filtration. Using a combination of polymer types and sizes, mussels were exposed to three environmentally relevant concentrations of MPs. Pseudofaeces, faeces and whole mussels were examined for MPs 24-hours post exposure. While whole mussels had significantly more MPs than pseudofaeces and faeces ($p<0.01$; mean proportions ranged from 46-68%, 2-4%, 3-8%, respectively) our results confirmed that MPs were both rejected prior to, and eliminated post digestion, suggesting that blue mussels might be a poor indicator of MP pollution. If plastic loads continue to increase as theorized, however, it is probable that the ability of blue mussels to reject and eliminate MPs efficiently will be impacted.

Keywords: microplastics; blue mussel; bioindicators; shellfish; marine pollution

To my Family & Friends

Acknowledgements

There are many people who have contributed to the completion of this thesis. I would like to extend a warm thank you to my supervisory committee, Dr. Leah Bendell and Dr. Peter Ross for the opportunity to conduct my own research in a supported environment. In addition, I would like to recognize my committee members Dr. Anne Salomon and Dr. Chris Harley who provided thoughtful input into the research and writing process. The projects completed for this thesis were funded by the Mitacs Accelerate Internship program, the Ocean Wise Conservation Association, Simon Fraser University, Fisheries and Oceans Canada (DFO) and the Port of Vancouver. I appreciate the opportunity and flexibility that came with this support.

Many hands, boats, cars and planes were involved in delivering, deploying and retrieving the thousands of mussels required to complete both the field and laboratory experiments required for this project. Thank you to the Tsleil-Waututh Nation and the Wei Wai Kum First Nation groups for providing access and local knowledge. In addition, many individuals provided their houseboats, docks, boat access and assistance in the depths of winter to house mussels for this project. Particularly, members of the Environmental Plastics Lab, Fisheries and Oceans Canada in Campbell River, Vancouver Island University, members of the Deep Bay Marine Field Station and the Vancouver Aquarium who were directly involved, thank you for your assistance. Without these collaborations and commitment to research, the caged mussel study would not have been a success.

Undoubtedly, the biggest support has always lied within the *'Brain Trust'* (members of the Environmental Plastics Lab at Ocean Wise), who provided continual support to stay on track and find the elusive plastic. In particular, thank you to Mégane Néauport for trialing the mussel digestions required for all aspects of this project, as well as Gabriela Aguirre, Nadine Trottier and Stephen Chastain who were instrumental in completing the mussel feeding experiment, along with the support of the Pacific Science Enterprise Centre for providing the facilities to house my mussels and complete my laboratory experiment.

I want to extend a heartfelt thank you to Marie Noël and Anna Posacka for their guidance in study design, and for ensuring I was able to obtain the many chemicals,

equipment and lab space required to complete my project work. To the wonderful women who have been by my side throughout this entire process, Rhiannon Moore, Lauren Howell and Ellika Crichton. Thank you for providing laughter, and light.

Lastly, thank you to my family for always encouraging me to chase my dream of working outside, under the sun in the absence of a path to get there. Your support has always meant I could dream as big as I wanted and never fear the possibility of failure.

And of course, we must acknowledge the hearty and ever-active Blue Mussel.

Table of Contents

List of Tables

List of Figures

List of Acronyms

ABS	acrylonitrile butadiene styrene
ANOVA	analysis of variance
BB	background blank
BCI	body condition index
BWW	body wet weight
CRD	complete randomized design
CSMP	corrected suspected microplastic
F	faeces
FTIR	Fourier transform infrared spectroscopy
IAP	instant algae paste
MP	microplastic
NP	nanoplastic
OAP	other anthropogenic particle
PA	polyamide (nylon)
PB	procedural blank
PC	polycarbonate
PE	polyester
PES	expanded polystyrene
PET	polyethylene terephthalate
PF	pseudofaeces
PP	polypropylene
SE	standard error
SF RCB	single-factor randomized complete block
SMP	suspected microplastic
TWW	total body wet weight

Chapter 1.

Introduction

1.1. Plastic Pollution

Plastic pollution is considered a ubiquitous contaminant within terrestrial and marine environments (Geyer et al., 2017). Prevalent within the modern world, plastic pollution is a proposed indicator for the 'Anthropocene' epoch of time due to continual deposition in terrestrial and marine sediments (Zalasiewicz et al., 2016). Mass production began in the 1950's with demand increasing exponentially (Boucher and Friot, 2017). An estimated 335 million tonnes of plastic was produced in 2016 (PlasticsEurope, 2017), with an estimated 8,300 million metric tonnes produced globally to date (Geyer et al., 2017).

Plastic pollution within the marine environment has been published in peer-reviewed journals since the 1970's (Jambeck et al., 2015; Mai et al., 2018). Mismanaged waste is considered the largest contributor of plastic pollution within our oceans, with the top five polluting countries located in southeast Asia (Jambeck et al., 2015). The term 'micro-plastic' wasn't established until 2004, however, after Plymouth university researchers embarked on a study to determine the smallest size-fractions of plastics observable within the marine environment (Thompson et al., 2004). In the following 14 years since publication, microplastic (MP) research has increased dramatically within the scientific literature (GESAMP, 2015; Lusher et al., 2016).

Plastic polymers 1 µm – 5 mm in diameter are categorized as a MP particle (Andrady, 2011; Shim et al., 2016), while fragmented plastics below 1 µm are considered nanoplastics (Gigault et al., 2018). The most common polymer groups manufactured include polyester (PE), polypropylene (PP), polyethylene terephthalate (PET), polyamide (nylon), poly-vinyl chloride (PVC), polystyrene (PS, including expanded polystyrene (EPS)), and cellulose acetate (Andrady, 2011; GESAMP, 2015; Hidalgo-Ruz et al., 2012; Rocha-Santos and Duarte, 2015). Each polymer has a specific density that is further altered by additives such as fillers, colourants and stabilizers (Andrady, 2011; GESAMP, 2015). Hence, MP fate within the marine environment is

heavily determined by polymer type, an important consideration when determining sampling collection and isolation techniques. Biofouling can also change particle density and alter settling patterns (Van Cauwenberghe et al., 2015b, 2015a).

MPs have been reported at polar ends of the Earth; within Arctic sea ice (Peeken et al., 2018), Antarctic seawater (Cincinelli et al., 2017), to the depths of deep sea sediments (Woodall et al., 2015, 2014), and within the atmosphere (Dris et al., 2015). Sources of MPs within the marine environment stem from wastewater effluent, derelict fishing gear, mismanaged waste, storm water drainage, industrial cleaners and atmospheric fallout (Andrady, 2011; Browne et al., 2007; Dris et al., 2015; Gies et al., 2018; Jambeck et al., 2015). Mechanical degradation (from wave action; (Jahnke et al., 2017)) aided by photo-oxidative degradation (from the sun) makes plastic brittle and prone to breaking (Andrady, 2017, 2011; Browne et al., 2007). This phenomenon, known as weathering, creates MP particles in an infinite combination of shapes, colours and sizes; making extraction and identification difficult (Hale, 2017).

MP contamination is now considered a global phenomenon with the potential to adversely affect marine organisms (Browne et al., 2007; Carr, 2017). Because MPs are so variable and difficult to identify, determining the deleterious effects to organisms is inherently difficult. Further research, using standardized techniques, is needed to understand MP uptake within species in both field and controlled laboratory experiments. This will allow researchers to better understand the adverse effects MPs pose to organisms, marine ecosystems and human health (Lusher et al., 2016; Smith et al., 2018).

1.2. Microplastic Identification

Visual microscopy is the most commonly used tool to identify MPs. Once identified, MP particles are categorized most often by shape and colour. Shapes include fibres, fragments, spheres (aka microbeads or pellets), films or foamed particles (Lusher et al., 2017; Mathalon and Hill, 2014). Colour definitions are project specific and help theorize particle origin and aid in identifying potential contamination sources during sample processing (Gago et al., 2018; Hidalgo-Ruz et al., 2012; Mathalon and Hill, 2014). Brightly coloured or unnatural colouration also aids in identifying anthropogenic particles among natural debris (Hidalgo-Ruz et al., 2012; Lusher et al., 2017, 2016).

Particles are identified using visual microscopy. Without polymer identification, however, misidentification of plastic particles can be as high as 70% (Shim et al., 2017).

Thus, visual microscopy should be supplemented with polymer identification on a subset of particles. It is suggested that a minimum 10% of all identified particles ranging in size of 100 µm – 5 mm, and all particles smaller than this be sent for polymer analysis (Lusher et al., 2016). The practicality of this is determined by resource availability and budget. The most common polymer identification techniques include Fourier Transform Infrared Spectroscopy (FTIR), Raman spectroscopy, scanning electron microscopy or pyrolysis–gas chromatography-mass spectrometry. Each technique poses unique advantages and disadvantages in terms of cost, sample loss and reliability of results (Gago et al., 2018; Lusher et al., 2017; Mai et al., 2018; Shim et al., 2016).

FTIR is the most commonly used technique (Gago et al., 2018) and is utilized throughout this study. An infrared beam excites particle molecules to create a spectrum that is then run against a library (Courtene-Jones et al., 2016; Shim et al., 2017). The lower size limit for this technique is 10 µm as this is the width of the scanning beam (Shim et al., 2017). Polymer identification is subjective as spectra are visually checked to confirm polymer type (Lusher et al., 2017). Preference for automated techniques (to reduce user bias) exists, however, the technology to analyze complex samples is currently lacking (Shim et al., 2016). Polymer identification results are used to correct initial plastic estimates and better reflect MP load in the marine environment (Catarino et al., 2018).

Difficulties also arise when identifying cellulose (considered natural) and rayon (considered semi-synthetic) as spectra are similar (Comnea-stancu et al., 2017). Rayon (known as viscose in Europe (Comnea-stancu et al., 2017)), is a heavily modified cellulose-based particle and researchers disagree on polymer categorization. Some studies treat rayon as plastic, others do not (Lusher et al., 2017; Peeken et al., 2018; Woodall et al., 2014). Weathering likely affects polymer identification and is a prominent data gap requiring further attention (Comnea-stancu et al., 2017).

1.3. Microplastics in Aquatic Organisms

MP abundances have been established in >200 marine organisms at all trophic levels (Lusher et al., 2016). Impacts to species and ecosystem health is still poorly understood (Duis and Coors, 2016; Hale, 2017). Potential impacts include false satiation, organ blockage and translocation to tissues (Browne et al., 2008; Lusher et al., 2013; Wright et al., 2013). MPs are most commonly found in the digestive tract (Smith et al., 2018), however, residence time is variable depending on the organism (Duis and Coors, 2016).

MPs can act as both a source and sink of harmful chemicals (Rochman et al., 2013) and is considered a threat to both animals and human health (Smith et al., 2018). Once ingested, it is theorized that MPs may leach additive chemicals or adsorbed persistent organic pollutants (POPs). The release of toxicants, however, remains a prevalent data gap that requires further research (Smith et al., 2018). Contributing to this, particles exhibit their own physicochemical properties and interact with biological organisms differently than well-studied chemical contaminants (Lambert et al., 2017; Potthoff et al., 2017; Qu et al., 2018).

Numerous exposure experiments have been conducted to understand the toxicity MPs pose on marine species. To date, exposure concentrations are highly elevated and most often utilize particles of one polymer type and shape (Browne et al., 2008; Cole et al., 2016; Farrell and Nelson, 2013; Kolandhasamy et al., 2018). Considered a prominent data gap, researchers have called for toxicity testing using environmentally relevant, multi-shaped MPs (Green et al., 2017; Lusher et al., 2016; Qu et al., 2018) to further define MP effects and provide regulators information for reduction targets and mitigation strategies.

1.4. Blue Mussel Physiology & Ecology

Filter feeding bivalves are integral components of nearshore ecosystems (Pales Espinosa et al., 2016). Individuals filter large volumes of water up to 12 hours each day (Van Cauwenberghe et al., 2015b). Water brought into the mantle cavity is initially, non-selective. That is, whatever is present within the water column is brought into the shell cavity and subsequently sorted. Filter-feeding bivalves therefore remove phytoplankton

from coastal ecosystems and aid in nutrient cycling through waste deposition (Pales Espinosa et al., 2016; van der Schatte Olivier et al., 2018). Seston quality is important in determining the filtration rate and assimilation of nutrients within individuals (Widdows et al., 1979).

Referred to as ecosystem engineers, blue mussels facilitate complex algae and invertebrate assemblages (Pfister et al., 2016) that increase biodiversity (Harley, 2011). Harvested for consumption and grown commercially, blue mussels are an important economic resource for coastal communities (Catarino et al., 2017; McPhee et al., 2017).

As they are an intertidal species, mussels are exposed to a range of environmental conditions (Helmuth et al., 2016). High zone individuals live at the extent of their thermal tolerances (Harley, 2011). Feeding is accomplished by filtering large volumes of water over the gill surface. Dependent on water quality parameters and seston concentrations, *M. edulis* can filter 0.39 L/hr/g under optimal conditions (Foster-Smith, 1975). This incurs a chronic, daily exposure to marine pollutants floating within the water column. Considering their feeding behaviour and sessile lifestyle, mussels face a myriad of anthropogenic pressures inclusive of climate change, ocean acidification, contaminants and most recently established, MPs (Gaylord and Al, 2015; Harley et al., 2006; Smith et al., 2006; Sunday et al., 2011).

The mussel watch program was established in 1975 to monitor marine contaminants using the blue mussel as a bioindicator (Goldberg, 1975). Given their high abundance, global distribution and ease of access, mussels are considered a low-cost monitoring tool of lipophilic and other contaminants (Giltrap et al., 2013; Luoma, 1996). Over 40 years later, the application of biomonitoring has spread globally (Farrington et al., 2016). This is because persistent organic pollutants, heavy metals and petroleum accumulate within the soft tissue of the organism at similar rates to concentrations found in the marine environment. These trends are assumed to also occur with MPs. For this reason, researchers have established the use of blue mussels as indicators of MP contamination in the surrounding environment (Catarino et al., 2018, 2017; Kolandhasamy et al., 2018; Qu et al., 2018).

MPs are well studied in marine mussels. Due to their ecological and economic importance, baseline MP contamination levels are being determined around the globe.

Researchers have established MP abundances in both wild and commercially grown individuals. A prominent study conducted on the east coast of Canada found >100 MPs/individual for both wild and farmed mussels. Similarly, it was previously estimated that European shellfish consumers ingest 11,000 MPs/year (Van Cauwenberghe and Janssen, 2014).

Exposure experiments have shown deleterious effects to blue mussels after acute exposure to high volumes of particles. Individuals exhibited necrosis, and reduced lysosomal membrane stability (Bråte et al., 2018; Browne et al., 2008; Von Moos et al., 2012). Although prominently cited in the literature, it is now being established that these early studies lacked rigorous contamination control and utilized highly elevated concentrations of MPs to establish effects. Greater attention is needed to expose organisms to environmentally relevant, multi-shaped plastics of differing polymer types.

More recently, researchers have established lower MP abundances within the blue mussel. When laboratory contamination is controlled, accounted for, and polymer identification follows, concentrations within individual mussels is significantly lower than originally reported. Most recently, a study along the coast of Scotland identified 3.2 (0.052 SE) MPs/mussel (3.0 (0.9 SE) MPs/gram of tissue) for *Mytilus edulis* (Catarino et al., 2018). Nile Red staining and FTIR were used to confirm polymer identify, revealing only 48 – 50% of identified particles were in fact plastic.

Similarly, a mesocosm experiment utilizing one of the lowest concentrations of MPs published to date (2.5 µg MPs/L and 25 µg MPs/L, as of December 2016), found that mussels exposed to MPs over a 50-day period exhibited no significant differences in sediment biodiversity or changes to cyanobacteria biomass. Comparatively, the flat oyster (*Ostrea edulis*) exhibited increased filtration rates, and a significant decrease in biodiversity and cyanobacteria biomass (Green et al., 2017).

Bioindicators are important resources to understand cumulative impacts of chemicals and changes to habitat (Holt and Miller, 2011). Often, bivalves (or other macroinvertebrates) are used as bioindicators to utilize the information gained through their filter feeding behaviour and accumulation of contaminants such as polychlorinated biphenyls (PCBs) or polycyclic aromatic hydrocarbons (PAHs) (Gadzała-Kopciuch et al., 2004). Considering these results, it is currently assumed that MPs extracted from the

digestive tract or whole body tissue correlate to the MP loads within the surrounding environment.

Research is needed to determine particle fate post mussel filtration and to quantify MP contamination rates within British Columbia blue mussels. A combination of field and laboratory techniques, when combined, can provide insight on the vulnerability mussels face when exposed to MPs, and the practicality of using the blue mussel as an indicator of MP pollution within the marine environment. Combined with a controlled laboratory experiment, using environmentally relevant concentrations of MPs with a variety of shapes and polymer types, researchers will further understand particle fate and the role blue mussels play in assessing marine MP pollution.

1.5. Thesis Objectives

Using rigorous contamination control, and polymer analysis, MP abundances in British Columbia waters will be determined. An exposure experiment using environmentally relevant, multi-shaped concentrations of MPs will be conducted in parallel, to assess impacts under controlled conditions. Together, the results of these studies will be used to assess if the blue mussel is a good indicator of MP pollution within coastal environments.

A caged mussel study (CMS) was established to determine if MP accumulation within the shell cavity of the blue mussel is quantifiable over a 60-day exposure period in the marine environment. At the start of this study, it was assumed that MPs accumulated within the shell cavity through adherence and/or ingestion within body organs. Mussels were placed in 11 locations of varying anthropogenic pressure to determine if MP accumulation differed by locality. For this reason:

> *H_0 = Mean MP abundance within the blue mussel (*Mytilus edulis*) shell cavity is equal between all sites and time periods when in the marine environment for a 60-day period.*
>
> *H_1 = Mean MP abundance in the blue mussel (*Mytilus edulis*) shell cavity increases between sites and time periods when in the marine environment for a 60-day period.*

The quantifiable findings from the CMS study will help researchers better understand the applicability of using the blue mussel as an indicator of MP pollution within coastal environments.

To further understand the CMS field-findings an exposure experiment will be conducted using environmentally relevant concentrations of MPs. At the time this study was completed (April 2018), the exposure concentrations were the lowest numbers exposed to any marine organisms (to the best of the authors knowledge). The intention of this study was to better understand particle fate post filtration.

This was accomplished by examining the pseudofaeces (PF), faeces (F) and mussel 24-hours post exposure. It is theorized that mussels filter seawater based on seston quality to maximize energy gained. This physiological response likely occurs after filtration when particles within the water (i.e. phytoplankton, organic matter and/or silt) are selected or rejected by the ctenidia or palps (Bayne et al., 1993; Pales Espinosa et al., 2016). Rejected material is expelled through the excurrent siphon as PF (appearing colourless and fluffy in low seston concentrations) while selected material is digested and subsequently rejected as faeces (appearing brown and pellet-like). Both PF and F will be sampled to determine if and how many MPs are present. Hence:

> *H_0 = Mean proportion of MPs within the PF, F and mussel does not differ between concentration groups 24-hours post exposure.*
>
> *H_1 = Mean proportion of MPs within the PF, F and mussel will differ between concentration groups 24-hours post exposure.*

Results from this experiment will be used to determine the proportion of particles within the three sample types (PF, F, mussel) to better understand if mussels are capable of rejecting (through PF), eliminating (through F) and/or retaining (within the mussel) MPs. This information will help assess the role of blue mussels as indicators of marine plastic pollution.

Chapter 2.

Microplastic accumulation within the shell cavity of British Columbia blue mussels (*Mytilus edulis*)

2.1. Introduction

It is currently theorized that marine MP load can be assessed using the blue mussel. Due to the mussel's indiscriminate feeding behaviour, exposure to MPs present in the marine environment presumably occurs each day. Filter feeding brings any particulate matter present in seawater into the shell cavity and across the gill surface to the mouth for digestion. Particle accumulation is assumed to occur through ingestion and/or adherence to body organs (Bråte et al., 2018; Catarino et al., 2018, 2017; Kolandhasamy et al., 2018).

A study conducted in the northeastern Pacific Ocean determined MP loads within the marine environment along southern British Columbia and along line P within the pelagic zone (Desforges et al., 2014). Subsurface (4.5m depth) concentrations ranged from 279 (±178 SD) – 7,630 (±1410 SD) particles/m^3. The mean for all sites was 2,080 (± 2,190 SD) with the most particles observed in a single sample being 9,180 particles/m^3. The authors noted that particle concentrations were 4 – 27 greater in nearshore sites compared to offshore locations within the Pacific Ocean. On average, particles were 606 (± 221 SD) µm in size. This data confirms that MPs are present within coastal BC waters.

Blue mussels are likely exposed to anthropogenic particles, including MPs, during the filter feeding process. This intertidal, coastal species is present along shorelines, and is often attached to pilings or floating docks. In British Columbia, three species of closely related blue mussels are found in the wild (*Mytilus trossulus, M. galloprovincialis and M. edulis,* (Schmidt, 1999)). Only *M. trossulus* is native to BC waters, while *M. edulis* and *M. galloprovincialis* were introduced. All species are known to hybridize, making identification impractical without genetic testing ((Heath et al., 1995)*). M. edulis*, however, is grown commercially on the west coast of Canada and was therefore used throughout this study.

MPs have been quantified within the blue mussel using various extraction techniques. Most often, individuals are purchased from a market or collected from the wild and immediately frozen for analysis (Lusher et al., 2016; Rochman et al., 2015). Values are reported as the number of MPs observed per individual and per gram of wet weight (to standardize for size (Catarino et al., 2018, 2017; Li et al., 2015; Lusher et al., 2016; Van Cauwenberghe and Janssen, 2014)). Generally, values reported for the number of plastics in mussels is <10 particles per individual (Catarino et al., 2018; Lusher et al., 2017; Mai et al., 2018; Renzi et al., 2018).

Due to the variety of sampling, extraction and reporting techniques described in the literature, researchers have put out a call to standardize methods globally to allow for inter-study comparisons at multiple scales (Amaral-Zettler et al., 2016; Catarino et al., 2017; Mai et al., 2018; Rochman et al., 2017). Extracting MPs from biological tissues is one of the challenges faced in achieving these goals. Published methods have utilized chemicals or enzymes to destroy biological tissues while leaving plastics within a sample unaffected. Acid digestion was initially a popular method used to extract MPs (Claessens et al., 2013; De Witte et al., 2014; Van Cauwenberghe and Janssen, 2014), followed by strong bases such as KOH (Foekema et al., 2013; Lusher et al., 2017; Rochman et al., 2015) and oxidation using H_2O_2 (Avio et al., 2015; Li et al., 2016; Mathalon and Hill, 2014; Qu et al., 2018).

Currently, the use of KOH and enzyme digestion are preferred extractive techniques as plastic polymers are not affected as biological tissues break down (Lusher et al., 2016). Furthering this, enzymes are effective at low temperatures (~50°C – 60°C) and do not melt plastics present within a sample. Until recently, however, enzyme digestion was considered a costly technique requiring expensive materials with small sample volumes being processed (Amaral-Zettler et al., 2016; Cole et al., 2014). In 2017, however, a method established by Catarino et al. utilized an industrial grade enzyme to digest mussel tissue and retain MPs present. This low-cost solution was proposed as a standardized technique to determine MP abundances within mussels globally and was subsequently utilized for this study program.

The study objectives of this report are two fold, 1) determine if MP accumulation is quantifiable within the shell cavity of blue mussels and 2) quantify MP abundances in British Columbia blue mussels. Utilizing mussels from one population source, individuals

were placed in cages at 11 BC locations and sampled over a 60-day period to determine changes to MP abundance through time and space. Combined, a comprehensive picture of MP accumulation within the blue mussel will emerge.

2.2. Materials and Methods

Cage Deployment and Sample Collection

Approximately 15, 000 blue mussels (*Mytilus edulis*) were received directly from an aquaculture farm (Taylor Shellfish Farms) in Powell River, British Columbia in January 2017 (Table 2.1). Individuals were part of a health assessment program to determine the accumulation of contaminants of concern over a 60-day period. Various urban sites were selected to quantify priority contaminants and determine potential implications to health and body condition. MPs were one of these priority contaminants measured and are solely reported in this document. Information on the other contaminants monitored will be reported in a separate manuscript.

Powell River is an active area for aquaculture and contains several sewage outfalls (RDN, 2018). Individuals received were roughly one year old and of similar size (mean shell length = 40 cm). It is therefore assumed that MPs were likely present within the shell cavity of the mussels used in this study. MP abundances in the mussels supplied from Powell River provide a baseline value (likely >0) for spatial and temporal comparisons of changes to MP abundances over time.

Long line mussels were collected from the aquaculture facility and individual mussel socks placed in cardboard boxes within a plastic bag. Boxes were immediately transferred via float plane to the Ocean Pollution Research Program laboratory (OPRP) in Vancouver, British Columbia. A subsample of individuals (n = 50) were frozen to determine baseline MP abundances within the shell cavity of each mussel (n = 8).

Mussels were gently removed from the socks in groups of 1 – 3 individuals and placed into clean Ziploc bags (n = 150 mussels / bag). Individuals were checked for prolonged gaping and/or lack of a startle response. Mussels exhibiting either of these behaviours were deemed unhealthy and removed from the study. Ziploc bags were placed unzipped, in open coolers for transport.

At each study location (n = 11) each bag of mussels was transferred into one of three tiers of a mesh lantern net (n = 150 mussels / tier). Mussels were placed in the top four tiers of the lantern nets (hereafter referred to as cages), while tiers not in use were zap strapped together to create a weighted bottom (cage length ~1m, cage width ~0.5m). Cages were composed of a black polyethylene, monofilament, woven raschel with a mesh size of 7 mm (MITACS, 2016). This mesh size allows particulates to float freely into the cage while preventing predation (Figure 2.2a). The tiers were woven together (by hand) using an orange, HDPE monofilament.

Mussels were shipped from Taylor Shellfish Farms on January 9, 2017 and deployed between January 9 – 11 (Table 2.1). Adverse weather conditions and delayed site permissions postponed cage deployment at the Howe Sound and Bowen Island locations. This required a second shipment of mussels (from the same source population) from Powell River to the OPRP lab on January 16, 2017. These mussels were stored in coolers overnight. Half were suspended at the Bowen Island site the following day (January 17, 2017). The Howe Sound site required boat access, and severe weather events delayed deployment until January 24, 2017. During the interim days (January 17 – 24) mussels were suspended in cages (n = 600 mussels / cage) in Horseshoe Bay, BC.

When possible, cages were hung with nylon rope at 1m depth on floating structures for ~60 days. Three cages were suspended in the water column at each site (except n = 2 cages at the Victoria and Howe Sound with 600 mussels / cage), and were generally placed 1 – 50m of one another. Given depth restrictions, two of the three cages at the Bowen Island site were hung at 0.5m depth. Cages at the Campbell River site were deployed 50 meters apart at 3m depth on a metal chain between two pilings. To account for tidal change and ensure 100% submergence, cages were hung on a rising 3.5m tide. As these were permanent structures, it is likely that cages were exposed at low, low tide periodically.

Two unique sites of note are the Howe Sound and Vancouver Aquarium locations (Figure 2.1). Mussels placed at the Vancouver Aquarium site were suspended from the ceiling into three settling tanks (Figure 2.2b). Raw seawater (piped in from Burrard Inlet) entered the settling tanks and subsequently drained into the filtration system. This site was chosen to assess MPs entering the facility. Mussels at the Howe

Sound site were deployed via divers for attachment onto two mooring buoys at the north and south end of Bowyer Island at 2.4 and 3.4m depth, respectively (Figure 2.2c). Submergence at 1m depth was not possible at the Howe Sound site due to buoy safety lines. Cages were approximately 1.8 km apart Figure 2.1.

Each site was revisited a second and third time between February 8 – 27 (between 25 - 32 days post deployment, T30), and March 9 - 7, 2017 (between 55 - 61 days post deployment, T60). Half of the mussels were removed at each sampling event (Table 2.1) and immediately frozen for MP analysis.

Microplastic Extraction from Blue Mussels

Mussels were processed in batches of 8 for each site and time period. Batches were thawed for 1 – 2 hours prior to MP extraction. All laboratory work was completed with glassware and metal dissecting tools inside a laminar flow cabinet or fume hood. A plastic squirt bottle was used to aid in rinsing equipment, however, the likelihood of particles shedding from the container is low.

Equipment was rinsed three times with filtered tap water (1 µm glass filter) and immediately used for processing. All personnel wore nitrile gloves and protective clothing to prevent airborne contamination from personal clothing. A 100% orange cotton jumpsuit was worn from January – December 2017. Orange suits were chosen as contamination fibres were easily detected and eliminated during visual microscopy. Due to the high number of cotton contamination fibres observed, however, lab personnel began wearing low shedding, yellow Tyvek jumpsuits (Dupont Tychem 2000) in January, 2018. No yellow contamination fibres were observed following this switch.

Batches were placed on a metal rack and covered with a glass dish inside of a laminar flow hood to thaw. Individual mussels were gently shaken to remove excess water within the shell cavity and total body wet weight (g) measured. Soft tissue was dissected away from the shell, placed into a 250 mL flask with 40 mL of filtered water with stir bar, and body weight was recorded (g). Flasks were immediately covered with tinfoil to prevent airborne contamination. Whole mussels were digested individually so that MP abundances could be enumerated within the shell cavity of each mussel. That is, any MPs present on the gill surface, adhered to the mantle or within the digestive

tract was retained and subsequently enumerated (all MPs identified were therefore assumed to be within the mussel shell cavity).

MPs were extracted from mussel tissue using an adapted method established by Catarino et al. (2017). Enzymatic digestion with Corolase 7090 (0.4 mL) was completed on a magnetic stir plate (150 rpm) overnight (55 – 61°C) for ~20 hours. The digestate was filtered using vacuum filtration onto a 20 µm, polycarbonate (PC) filter paper. Filter papers were transferred to a plastic petri dish with lid to dry.

A procedural blank was completed with each mussel batch to monitor for reagent and airborne contamination in the absence of a sample. A background blank was run simultaneously with each procedural blank to monitor for airborne contamination. For the background blank, a 20 µm filter paper was placed inside a rinsed petri dish and exposed to air during dissection/filtration and closed when batch samples were no longer exposed to air.

A total of 200 mussels were processed to determine body condition index (BCI) and quantify SMP abundances within the shell cavity of each mussel (n = 8 musses per site/time period). Digestate for 29/200 mussels could not pass through the 20 µm PC filter paper resulting in sample loss. Nine mussels came from the Bowen Island site (2 from T30 and 7 from T60), hence BI was removed from the analysis. Because of this, data analysis compared mean abundances at 11 sites, compiled from 171 mussels (n = 5 – 8 mussels per site/time period).

Suspected Microplastics

Visual microscopy (Olympus SZX16 (up to 18.4X) microscope with Olympus DP22 camera and DP2-SAL software, Olympus Corporation, Tokyo, Japan) was completed for each batch of samples (representing a single site/time period). Filter papers remained inside the petri dish with the lid on during microscopy to avoid contamination fibres from landing onto the sample. A coloured grid was used to systematically examine the filter paper (Figure 2.3). Particles exhibiting a minimum three of the following characteristics were identified as a 'Suspected Microplastic' (SMP, adapted from (Hidalgo-Ruz et al., 2012; Lusher et al., 2016; Marine & Environmental Research Institute, 2015):

- Absence of cellular or organic structures
- Homogeneous colour and brightness
- Unnatural colour and/or shininess
- Constant thickness of fragments and fibres
- Equal roundness throughout the entire length of fibre
- 3-dimensional bending of fibres

When a SMP was observed, the particle was photographed, measured and numbered. Particles were numbered from 1 – 'X' on a per batch basis. That is, the number sequence continued for all particles identified in mussels 1 – 8. Particle shape was also defined and binned into one of the following categories (adapted from (Hidalgo-Ruz et al., 2012; Lusher et al., 2016; Marine & Environmental Research Institute, 2015):

- **Fibres** – thin and round
- **Fragments** – three-dimensional with irregular shape
- **Films** – flat, exhibit a two-dimensional shape
- **Foamed** – air pockets present
- **Spheres** – nurdles and/or microbeads

Polymer Identification

A random selection of particles identified in each batch of mussels and all procedural blanks (20%) were sent to the Fourier Transform Infrared Spectrometer (FTIR) for polymer identification. SMPs were manually affixed to a glass microscope slide covered with a thin layer of 2% dextrose (Sigma-Aldrich, St Louis, USA). SMPs were analyzed using a Cary 670 Fourier Transform Infrared Spectrometer equipped with a Cary 620 microscope (Agilent Technologies, Mulgrave, AUS) using the micro-ATR accessory equipped with a germanium crystal.

Each background and sample scan ran128 co-added scans at a resolution of 8 cm^{-1} in the range of 3800 to 900 cm^{-1}. FTIR imaging spectra were matched against a commercial polymer database with 250,000 entrees (KnowItAll, BioRAd) of selected

ATR-FTIR spectra from polymers, plastics, polymer additives, plasticizers and packing materials. Sample spectra was identified successfully if the following criteria were met: i) all peaks were present in both reference and sample spectra (but peak intensity could vary) and ii) a total percent of the reference and sample spectra overlap was >70%.

Anthropogenic particles exhibiting two or less of the plastic qualities were ignored as they were not considered a SMP. This would include particles that looked anthropogenic but had frayed endings, irregular colouration and/or uneven widths (predominantly seen in cellulose based fibres). To validate these assumptions, 19 mussels (11% of total mussels examined) were examined for 'Other Anthropogenic Particles' (OAP). One quarter of each mussel (i.e. one quarter of the filter paper) was randomly selected and all anthropogenic, non-SMP particles enumerated. A subset of OAP particles identified (10%) were sent to the FTIR for polymer identification to confirm items were not plastic.

Data Analysis

BCI was calculated by comparing body wet weight to total wet weight (Bråte et al., 2018; Brooks et al., 2015). Mean BCI was established for each site and time-period (n = 5 - 8). A two-factor completely randomized analysis of variance (2F CRD ANOVA) was completed to analyze difference in mean BCI across time and space (both random effects).

$$\boldsymbol{BCI = \frac{body\ wet\ weight}{total\ wet\ weight}}$$

SMPs were corrected to account for contamination identified in procedural blanks. The mean number of SMPs / procedural blank were subtracted from the total SMPs observed for each mussel. The resulting 'Corrected Suspected Microplastic' data (CMSP) was further adjusted by replacing (-) values with 0's (a negative number of MPs within a mussel cannot occur), to ensure a realistic value of SMPs per mussel resulted. Data was converted to the #CSMP / gram wet weight (GWW) to standardize for differences in mussel size. The log10 (#CSMP/GWW +1) was calculated prior to analysis to decrease the standard deviation in mean abundance between groups (Figure 2.4).

To meet the factorial design of the ANOVA, values of CSMP/GWW from the T0 – Powell River site were repeated in the data set to allow for comparison at all 3 time periods. This was done for analysis and graphing purposes. A Tukey-Kramer multiple comparison test was completed to determine where the differences in mean CSMP/GWW occur. Analysis was completed using the statistical program JMP 13.0.0 (JMP, 13.0.0)

Table 2.1 Mussels were deployed at 11 sites within the Strait of Georgia, British Columbia, Canada for 60 days. Cages were hung at 1m depth (n = 450/cage) on floating or stationary structures from January 11 – March 27, 2017.

Site	Acronym	Deployment Structure	Structure Type	Cages Deployed	Cages Removed	Latitude	Longitude
Powell River	PR	NA*	NA*	NA*	NA*	50.063215°*	-124.661183°*
Campbell River	CR	Metal Chain	Stationary	January 11	March 14	50.039367°	-125.242933°
Deep Bay	DB	Marina Dock	Floating	January 11	March 14	49.464550°	-124.728367°
Victoria	VIC	House Boat	Floating	January 11	March 15	48.422967°	-123.382680°
Vancouver Aquarium	VA	Settling Tank	Stationary	January 9	March 9	49.300899°	-123.130641°
Coal Harbour	CH	House Boat	Floating	January 9	March 11	49.291004°	-123.124508°
Canada Place	CP	Marina Dock	Floating	January 9	March 11	49.286959°	-123.110903°
Port Moody	PM	Marina Dock	Floating	January 9	March 12	49.292516°	-122.891041°
Indian Arm - Mid	IM	Personal Dock	Floating	January 9	March 10	49.351433°	-122.908450°
Indian Arm - North	IAN	Personal Dock	Floating	January 9	March 10	49.461382°	-122.890604°
Bowen Island	BI	Marina Dock	Floating	January 17	March 20	49.379899°	-123.331153°
Howe Sound	HS	Mooring Buoy	Floating	January 27	March 27	49.432167°	-123.275200°

Note* Mussels hung from longline at aquaculture facility and immediately frozen upon arrival at the lab. Coordinates provided are approximate.

Figure 2.1 **Mussels were received from Taylor Shellfish Farms in Powell River (star) and deployed in cages at 11 locations (n = 450 mussels/cage), at 1m depth for ~60 days between January 11 – March 27, 2017. Mussels were collected on Day 30 and Day 60 to determine if MP accumulation is observable within the shell cavity of blue mussels over a 60 day period (Google Earth 2018).**

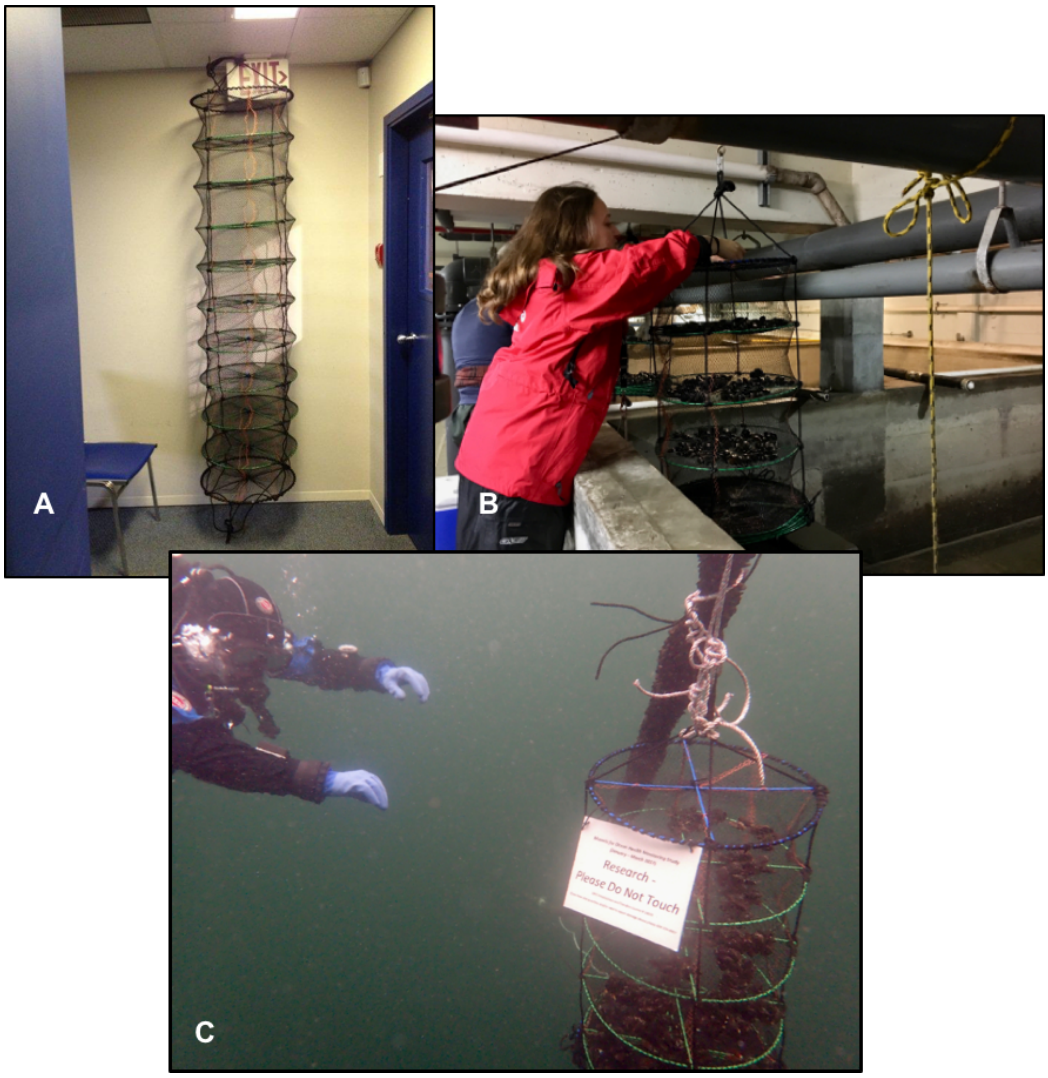

Figure 2.2 **Mussels were deployed in cages within the top three tiers of each net (photo A, Mitacs, 2016) for a ~60 day period. Cages were suspended from floating and stationary structures. Two unique sites included the the Vancouver Aquarium settling tanks (photo B) and the Howe Sound site, where two cages were hung from a bouy mooring line (photo C).**

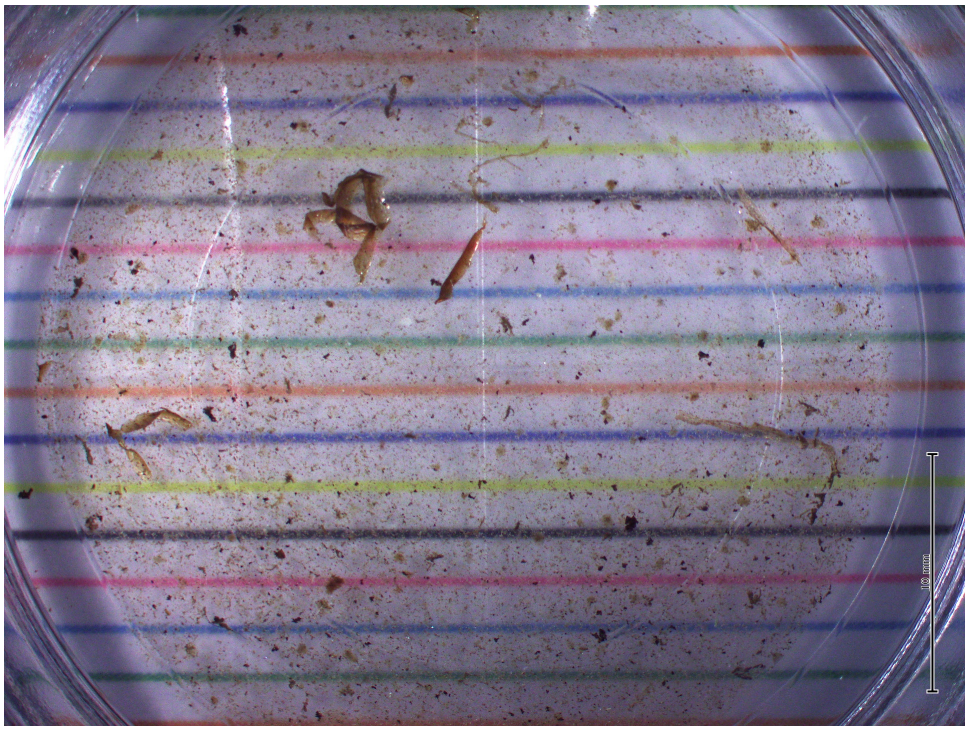

Figure 2.3 **Filter papers were examined for suspected microplastics using a coloured grid. Particles were observed among remnant biological tissue inside a plastic petri dish. Lids remained on during enumeration to ensure airborne fibres did not contaminate the samples.**

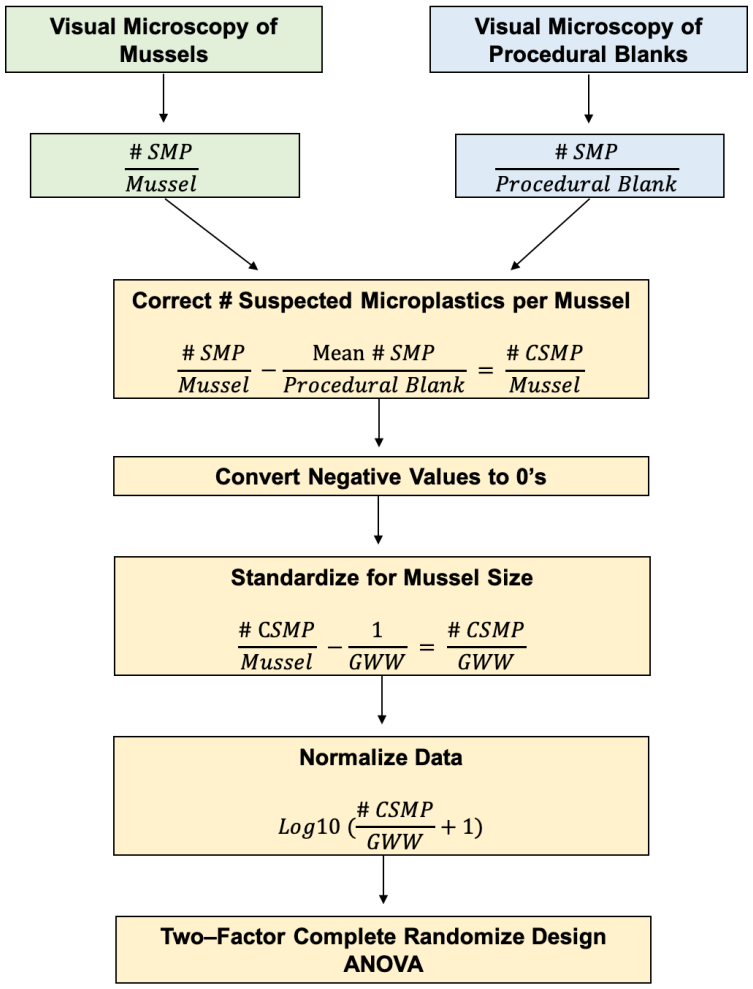

Figure 2.4 **The number of suspected microplastics (SMPs) observed in each mussel was corrected to account for the mean number of contamination fibres observed in 23 procedural blanks. Negative corrected values were further adjusted to zero. Individual values were then standardized for mussel weight and the data was normalized using log10 +1. A two-factor complete randomized design analysis of variance was completed to determine if the mean number of CSMP/GWW differed between the 11 sites over the 60 day period.**

2.3. Results

Mussel Length & Body Condition Index

Mean mussel length (cm) for T0 mussels (n = 7) was 39.5 cm (0.23 SE). Mean length increased to 42.9 cm (0.24 SE) for T30 individuals (n = 82). This 8.6% increase in growth was not observed when comparing the mean length of mussels at T30 to T60 sampling periods. During the final 30 days of deployment, mussels did not increase in size as mean length for T60 mussels was again 42.9 cm Figure 2.5. It is noted, however, that similar sized mussels were selected to prevent bias in the number of MPs observed in mussels between groups.

Mean BCI ranged from 35.9 (T30 – Coal Harbour) to 51.9 (T60 – Indian Arm – North). Overall mean BCI for T0 = 43.8 cm (0.13 SE; n = 7), T30 = 42.3 (0.4 SE; n = 83), T60 = 46.8 (0.38 SE; n = 76) across all sites. Mean BCI from T0 – T30 decreased by 1.5, while an increase in mean BCI was exhibited in the 30-day period when T30 and T60 mussels were sampled (increase = 4.5; Figure 2.6).

The 2F CRD ANOVA found evidence of an interaction of site and time period on mean BCI (F-statistic = 2.69, p-value = 0.0002). The Tukey Kramer comparison test revealed that mean BCI did not differ within an individual site (comparing T30 and T60 mean BCI at one location), but mean BCI did differ between sites/time periods. Mean BCI for T30-Howe Sound, T60 – Deep Bay, T60 – Port Moody, T60 – Indian Arm – Mid, T60 – Indian Arm – North and T60 – Howe Sound were significantly greater than mean BCI for T30 – Powell River. No other significant differences in mean BCI for mussels from T0 and T60 in Powell River were significant.

Abundance of Suspected Microplastics

In total 336 SMPs were quantified in 171 mussels resulting in a mean of 1.96 SMP/mussel (0.13 SE). Of the particles identified, 91% were fibres (n = 307), 6% fragments (n = 20), 2% spheres (n = 5), and 1% foam (n = 4). No film particles were seen. Dark (31%) and blue (29%) SMPs were most commonly detected. No yellow particles were observed in the study (Table 2.2).

Particles varied in size and shape across all time periods and sites. Fibres ranged in length from 29 µm – 9.0 mm with a mean length of 735 µm (57.1 SE). The mean diameter for all fibres was 20.3 µm (4.78 SE). A total 37 non-fibre particles were observed, 20 of which were fragments. Fragments had a mean length of 33, 320 µm (33,175 SE) and a mean width of 70.1 µM (20.7 SE). The largest particle observed within the mussel shell cavity was a green fragment (diameter = 597,307.7 µm; area = 46,201 μm^2). Note that the large size of this fragment heavily influenced the mean diameter of fragments observed, when excluded mean fragment length decreased to 145 µm (62.4 SE).

The smallest particle observed within the mussels was a blue sphere (diameter measured 31.3 µm by 26.0 µm). It is noted that the lower limit for quantifying particles was 25 µm. Particles < 25 µm were not clearly visible under the dissecting microscope and were unable to be properly inspected for SMP characteristics.

In total, 29 SMPs were observed in 23 procedural blanks. The number of SMPs ranged from 0 – 4 per filter, resulting in a mean of 1.26 SMP/procedural blank (0.26 SE; Figure 2.7). In total, 27 fibres, 1 blue fragment and 1 green fragment were observed. Fibres are noted to be the dominant contamination source (93%). Particles were observed in six colour categories, indicating that contamination sources were variable throughout the study (transparent (n = 8), dark (n = 7), blue (n = 5), green (n = 3), orange (n = 3), and red (n = 1)).

Mean SMP abundances were corrected by subtracting 1.26 particles from number of SMPs enumerated in each mussel. This resulted in 150 of the 171 mussels having "0" particles observed (Appendix A, Table A2 – yellow columns).

After standardizing for mussel size, the mean number of CSMP/GWW was 0.43 (0.06 SE). The data exhibited a non-normal distribution per group (batch of 8 mussels). Taking the [log10 (CMSP/GWW + 1)] reduced variation between treatment groups to allow the 2F CRD ANOVA to proceed. Note that after correcting for contamination, T0 – PR had only one mussel with any observed MPs. Therefore, the box plot for T0 is a line in Figure 2.8.

A 2F CRD ANOVA confirmed that there was an effect of the interaction of site and time period on mean CSMP/GWW abundance (F-statistic = 2.7, p = 0.0003). Only

comparisons made between mean abundances for T60 – Powell River differed significantly (Figure 2.9). This difference was significant for all comparisons made. A total of 26 comparisons were completed, resulting in p-values ranging from <0.001 – 0.021 with all confidence intervals excluding 0. The other 502 comparisons between all other site/time periods resulted in p-values >0.05, indicating no significant difference in mean abundance of CSMP/GWW was observed. Mean abundance did not differ within sites (i.e. comparing means at T30 and T60 for the same location) or between sites (i.e. comparing means of different sites) for all locations except Powell River at T60.

Polymer Identification

A total 66 SMPs and 31 OAPs were sent for polymer identification via FTIR. Of the SMPs, 43 originated from mussels, 13 from procedural blanks and 10 from background blanks (Table 2.3). Results as of July 2018 indicated that, in total, 11 of the 66 SMP particles were identified as plastic (n = 8 in mussels, n = 1 procedural blank, n = 2 background blanks). Of the SMPs confirmed plastic, 6 were PE, 2 acrylic, 1 nylon and 1 PET. The remaining 55 SMPs were identified as non-plastics, including cotton, wool, rayon and other semi-synthetic materials (Table 2.3). This gives a false positive rate of 83% when identifying plastics using the criteria described in section 2.2. Currently, it is not possible to determine synthetic cellulose, based on the preliminary results from a weathering study conducted within the Ocean Wise Environmental Plastics Lab. Further work may provide insight in the future. These results confirm it is inherently difficult to correctly identify plastic from synthetic particles using visual microscopy alone.

To confirm ignored OAP particles were not plastic, ¼ of a subset of mussels (n = 19) and all procedural blanks (n = 23) were enumerated for non-plastic particles and 10% were sent to FTIR for identification. In total, 50 OAPs were observed in the 19 mussel quarters examined and 47 in the quarter of all procedural blanks (n = 23). The mean number of OAP particles per ¼ mussel was 2.6 (0.84 SE) and a mean 2.2 OAPs (.50 SE) per procedural blank. Removing the mean number of OAP contamination particles observed in procedural blanks, the mean value of OAP particles observed per mussel ¼ falls to 0.4 OAP/quarter mussel. Multiplied by 4, the mean number of OAPs observed per mussel is 1.6. When including SMPs, the total anthropogenic particles observed in each mussel (SMP + OAP) becomes an average of 2.33 particles per individual.

A total 31 OAPs (32%) were sent to the FTIR to confirm the particles were not plastic. Of these, 1 particle was identified as plastic (acrylic), and the rest were deemed non-plastic. This incurred a false negative rate of 3%, confirming that 97% of all particles considered OAPs were non-plastic polymers.

Table 2.2 **Microplastics were divided into categories based on colour and shape. A total of 336 suspected microplastics (SMPs) were observed in 171 mussels. Of the particles identified, 91% were fibres. No film particles were observed. Particle colour was varied, with the most observed colour category being dark (31%) and blue (29%). No pink particles were observed. Grey columns depict particle shapes (n) observed per site/time period. Total number shapes (n) and percentage provided for all observed SMPs in bottom row. White columns detail observed SMPs colour (n). Note that frag = fragment, transp = transparent, comp = composite.**

Site	Time Period	Particle Shape					Particle Colour										
		Fibre	Frag	Foam	Film	Sphere	Dark	Blue	Red	Orange	Green	Purple	Pink	Brown	Transp.	White	Comp
Powell River	T0	7	0	0	0	0	4	2	0	0	1	0	0	0	0	0	0
	T30	9	1	0	0	1	2	3	2	0	2	0	0	1	1	0	0
	T60	22	5	0	0	3	6	11	5	4	0	1	0	0	2	0	1
Campbell River	T30	12	0	0	0	0	6	1	0	1	4	0	0	0	0	0	0
	T60	9	1	0	0	0	1	3	0	2	4	0	0	0	0	0	0
Deep Bay	T30	18	3	1	0	0	5	7	1	1	4	1	0	0	1	1	1
	T60	4	1	0	0	0	0	2	0	1	1	1	0	0	0	0	0
Victoria	T30	17	1	1	0	0	3	9	1	1	2	0	0	0	2	1	0
	T60	16	0	0	0	0	6	7	2	0	1	0	0	0	0	0	0
Vancouver Aquarium	T30	5	1	0	0	0	2	2	1	0	0	0	1	0	0	0	0
	T60	13	0	0	0	0	4	3	2	0	1	0	0	0	3	0	0
Coal Harbour	T30	10	0	0	0	0	4	3	2	1	0	0	0	0	0	0	0
	T60	24	3	0	0	1	10	16	0	1	0	0	0	0	1	0	0
Canada Place	T30	15	0	0	0	0	8	2	2	0	2	0	0	0	1	0	0
	T60	23	1	0	0	0	11	6	3	0	1	1	0	0	2	0	0
Port Moody	T30	13	0	0	0	0	3	1	3	0	1	0	0	0	4	0	1
	T60	22	1	2	0	0	7	4	3	6	1	0	0	0	2	2	0
Indian Arm - Mid	T30	21	0	0	0	0	7	4	5	1	1	1	0	1	1	0	0
	T60	9	1	0	0	0	2	3	2	0	1	0	0	0	2	0	0
Indian Arm - North	T30	13	0	0	0	0	4	4	0	3	2	0	0	0	0	0	0
	T60	8	0	0	0	0	6	0	0	0	0	1	0	0	1	0	0

Howe Sound	T30	12	1	0	0	0	2	5	2	1	1	0	0	0	1	0	1
	T60	5	0	0	0	0	2	1	1	0	0	0	0	0	1	0	0
Total All Sites/ Time Periods		307	20	4	0	5	105	99	37	23	30	6	1	2	25	4	4
		91%	6%	1%	0%	2%	31%	29%	11%	7%	9%	2%	1%	1%	7%	1%	1%

Table 2.3 **In total, 97 particles were examined using Fourier transform infrared spectroscopy to determine polymer type. 66 particles were suspected microplastics (SMP), and 31 were assumed not plastic (referred to as Other Anthropogenic Particles (OAP)). Particles were identified in either a mussel, procedural blank or background blank sample. Only 17% of the SMPs were identified as plastic (n = 11). All OAP particles, except 1 (3%), were correctly identified as non-plastic.**

Predicted Category	Sample	N	Plastic				Non-Plastic
			PE	Nylon	Acrylic	PET	
SMP	Mussel	43	3	1	3	1	35
	Procedural Blank	13	1	0	0	0	12
	Background Blank	10	2	0	0	0	8
	Sub-Total	**66**	**6**	**1**	**3**	**1**	**55**
		100%	**9%**	**2%**	**5%**	**2%**	**83%**
OAP	Mussel	14	0	0	1	0	13
	Procedural Blank	17	0	0	0	0	17
	Sub-Total	**31**	**0**	**0**	**1**	**0**	**30**
		100%	**0%**	**0%**	**3%**	**0%**	**97%**
TOTAL		97	6	1	4	1	85

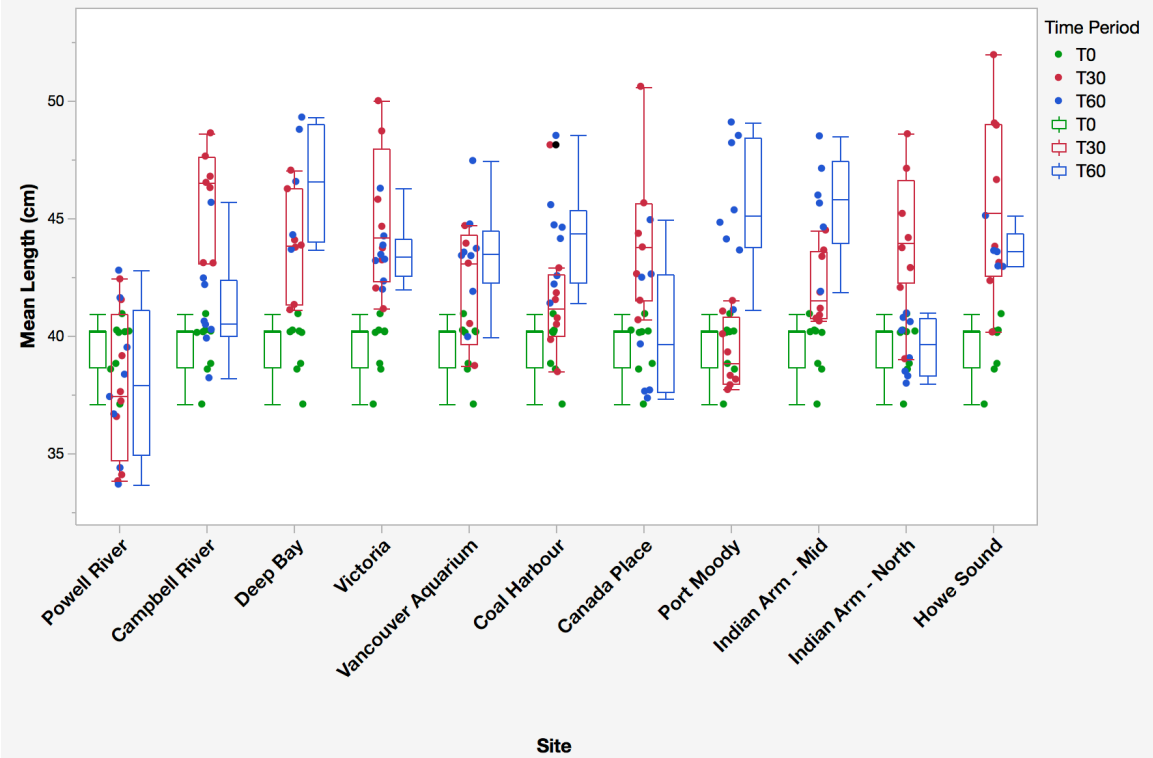

Figure 2.5 **Individuals sampled at T0, T30 and T60 had a mean length (cm) of 39.5 (0.23 SE), 42.9 (0.24 SE) and 42.9 (0.25 SE), respectively. Mean shell length between T0 (n = 7) – T30 (n = 82) increased by 8.6%. Mussels sampled at T30 and T60 (n = 76), however, did not exhibit a change in mean shell length. It is noted that mussels of similar size were selected for dissection to prevent bias in the number of suspected microplastics obsereved.**

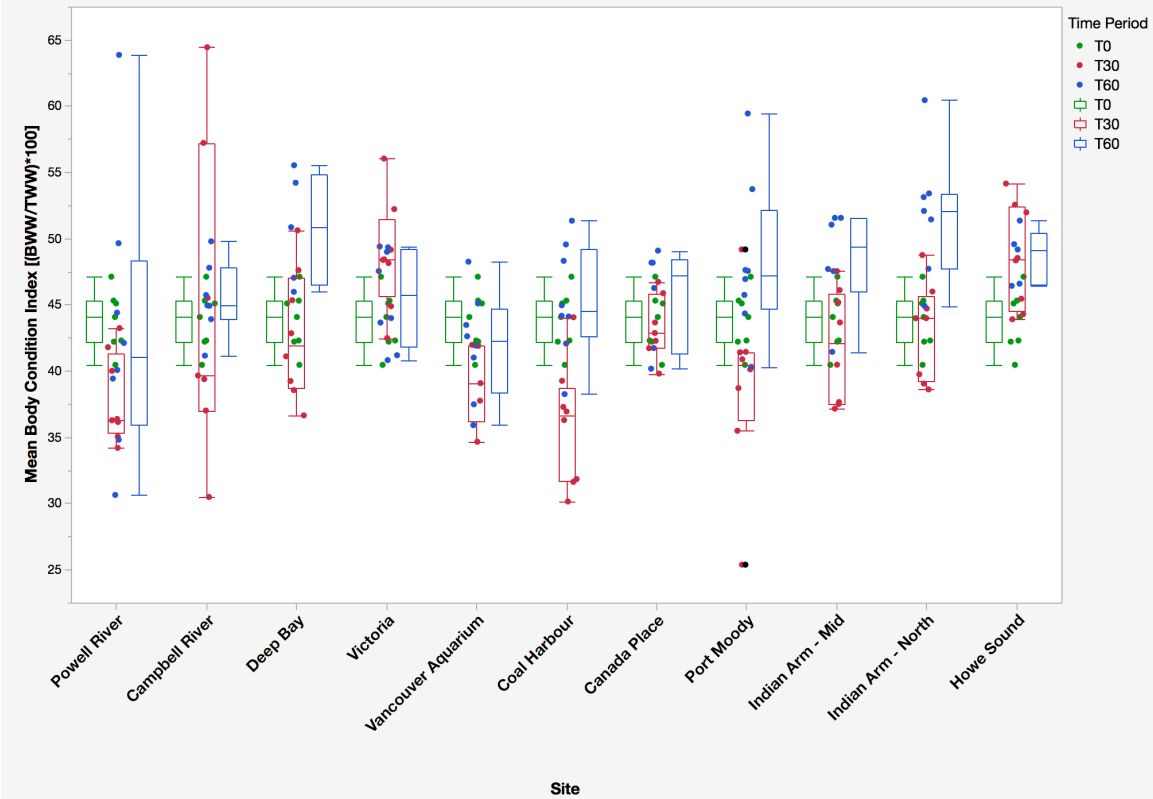

Figure 2.6 **Mean body condition index (BCI) for mussels (n = 5 – 8) at 11 sites across 3 time periods (T0 = green, T30 = red, T60 = blue) ranged from 35.9 (T30 – Coal Harbour) to 51.9 (T60 – Indian Arm – North). Compared to T0 mussels, T30 individuals exhibited a decrease in mean BCI by 1.5 (mean = 43.8 (0.51 SE), 42.3 (0.50 SE), respectively). Mean BCI for T60 individuals increased to 46.8 (0.52 SE) compared to T30 mussels sampled (increase = 4.5).**

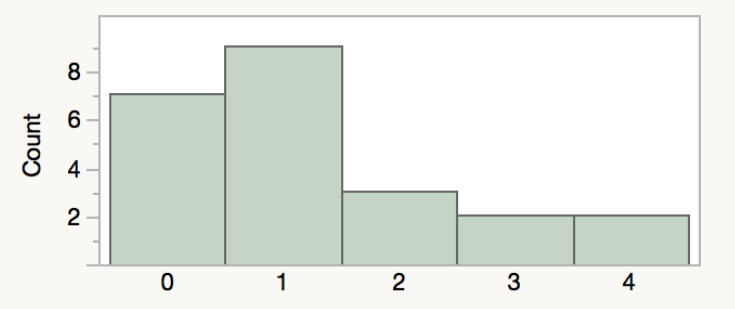

Figure 2.7 **The number of suspected microplastics (SMPs) observed on a procedural blank ranged from 0 – 4. One contamination particle per procedural blank was observed most frequently. Of the 13 SMPs sent for polymer confirmation only 1 was identified as plastic (8%) indicating that the majority of SMP contamination fibres were not plastic particles.**

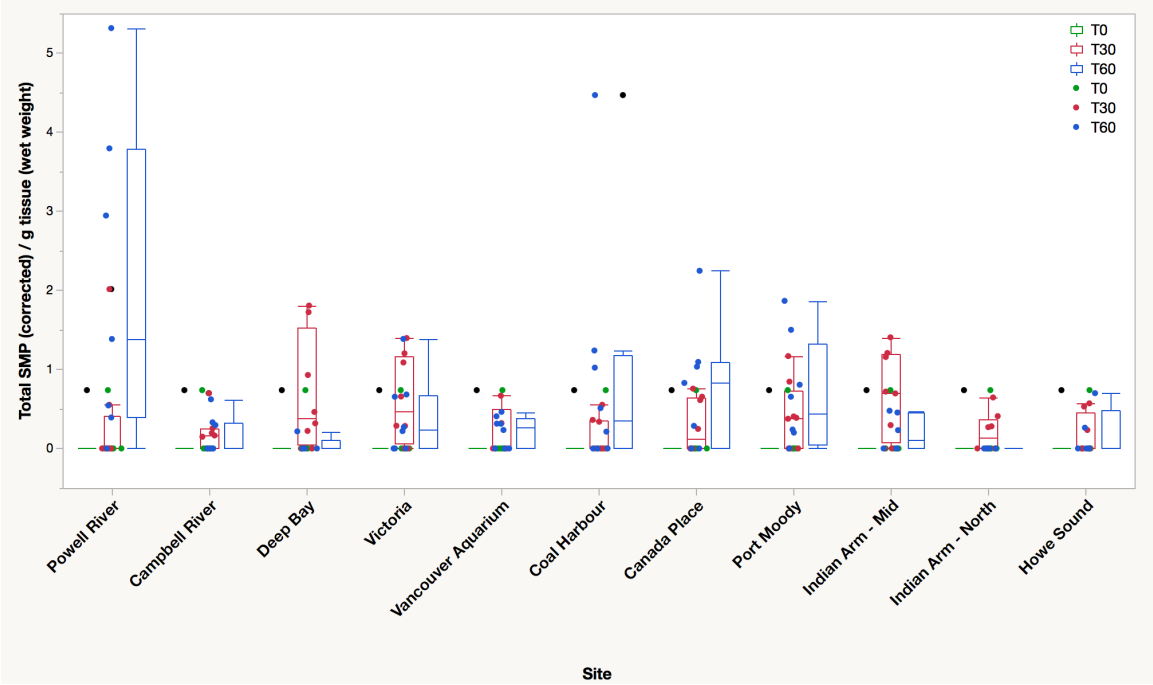

Figure 2.8 **Baseline abundances for the T0 – Powell River site are provided in green. Only one of seven mussels examined were found to have any suspected microplastics (SMP), hence the boxplot for T0 is a line. The T60 – Coal Harbour site had one mussel with 4.46 corrected SMPs per gram of wet weight (gww) and is considered an outlier. On average, mussels exhibited 0.43 (0.06 SE) corrected SMPs/GWW.**

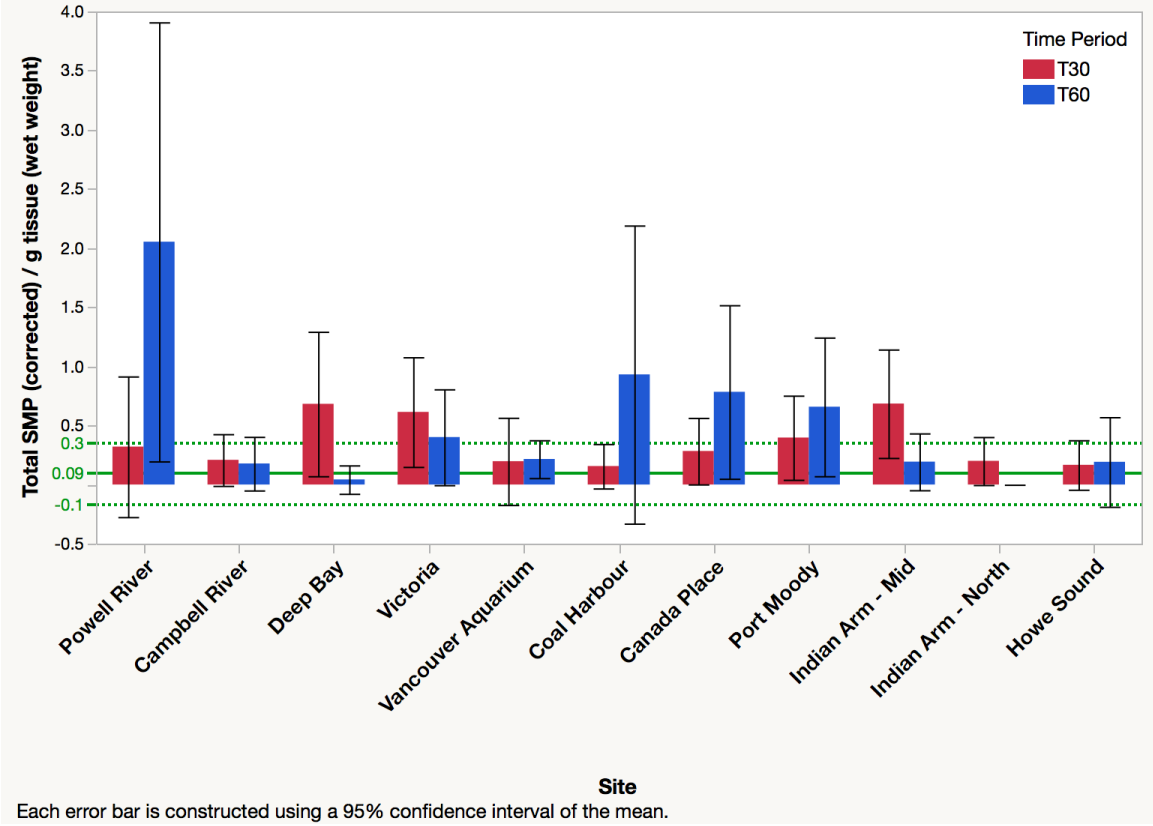

Figure 2.9 **Mean corrected suspected microplastics (CSMPs) per gram of wet weight (GWW) for T0 – Powell River mussels was 0.09 (0.06 SE), represented as a green line with 95% confidence interval (dashed lines). The difference in observed particles between sites and time periods was significant (p = 0.0003), however, only mussels at T60 – Powell River exhibited significantly more CSMPs/GWW. Overall trends indicate that mussels did not accumulate microplastics over a 60-day period.**

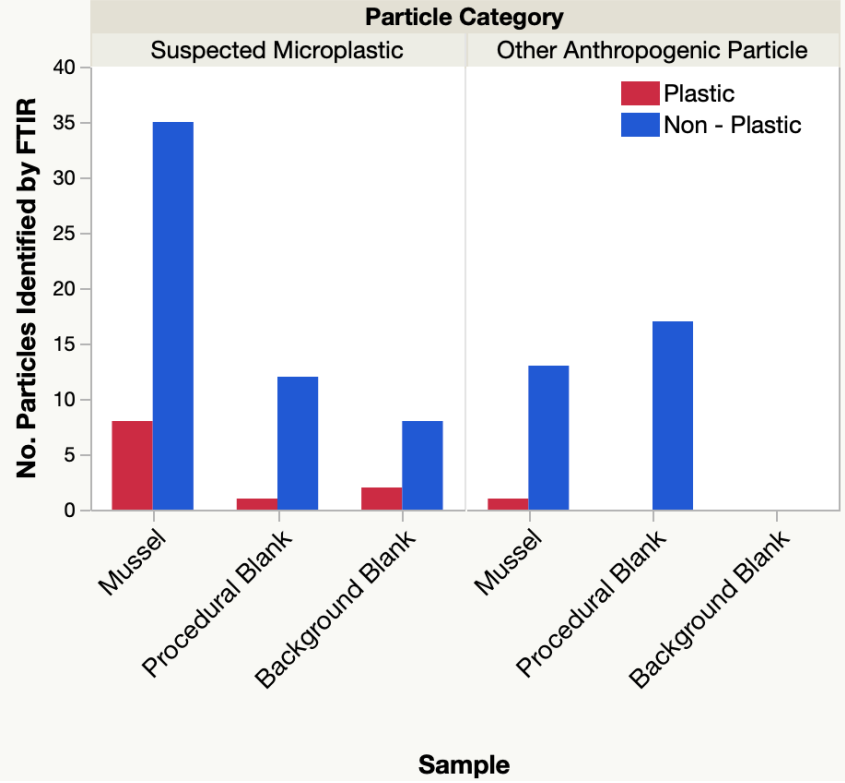

Figure 2.10 **A total of 66 suspected microplastics (SMPs) were analyzed by Fourier transform infrared spectroscopy (FTIR) to determine polymer type. In total, 11 of these were plastic (17%). 31 other anthropogenic particles (OAPs) believed to be natural or semi-synthetics were also analyzed to confirm that ignored particles were not plastic. Of those analyzed, one particle was identified as an acrylic plastic (3%). Particles confirmed to be non-plastic were a combination of cotton, wool, rayon and other semi-synthetic materials.**

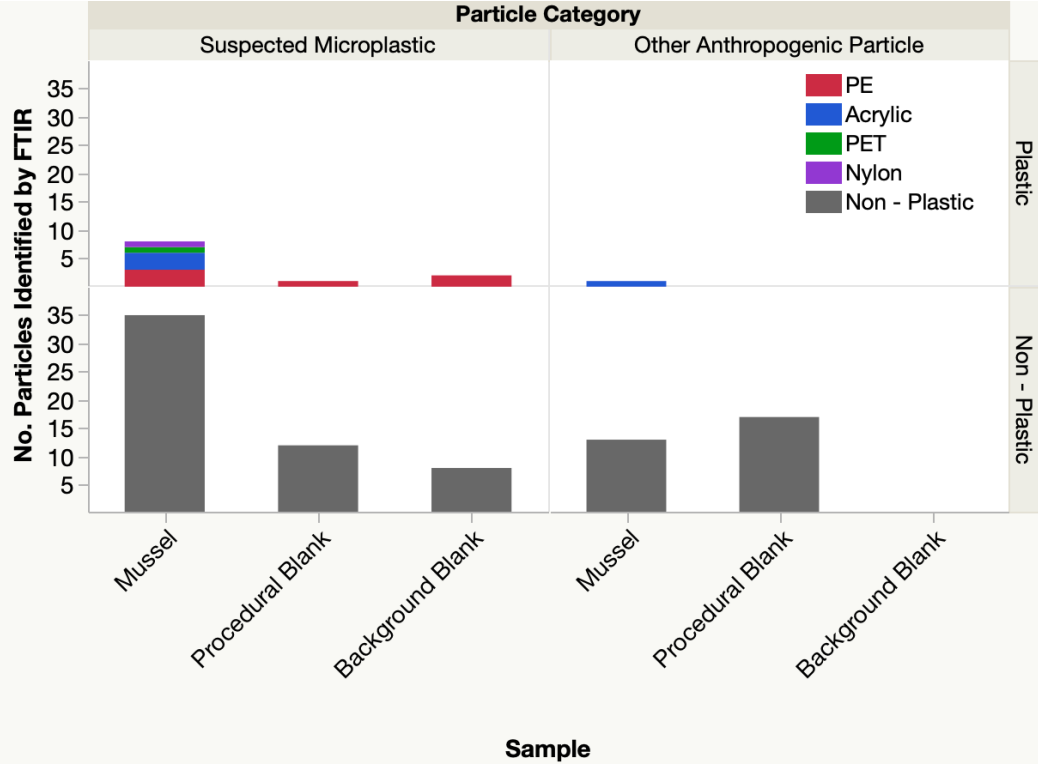

Figure 2.11 **Of the 66 suspected microplastics (SMPs) sent for Fourier Transform Infrared Spectroscopy (FTIR), only 11 were identified as plastic. Polymer types identified included polyester (PE), nylon, acrylic and polyethylene terephthalate (PET). Other anthropogenic particles (OAPs) were assumed to be non-plastics. 31 of these were sent to FTIR for confirmation and one was determined to be acrylic (3%). The remaning 30 OAP particels were confirmed non-plastics (97%). Particles deemed non-plastic included cotton, wool, rayon and other semi-synethic materials.**

2.4. Discussion

This study is one of the first to rigorously address external contamination, combined with the proper identification of MPs (25µm – 5mm) retained by the blue mussel within British Columbia. Of the particles recovered 91% were fibers. In total, 66 SMPs were sent for polymer identification by FTIR, of which, 11 (17%) were identified as plastic. Analysis comparing the mean number of SMPs per individual mussel and per GWW did not differ significantly between sites for all time periods. The only exception to this is T60-Powell River (n = 8), which had significantly more SMPs observed.

Mean BCI decreased slightly between T0 (n = 7) to T30 (n = 82) while mean mussel length increased (from 39.5cm to 42.9 cm). It is likely that changes to BCI during the T30 sampling period likely reflect differing anthropogenic pressures (i.e. pollution), variations in food supply and changes in reproductive status. The 30 days following this, mean BCI increased (by a factor of 4.5; n = 76), while mean mussel length remained constant (42.9 cm). Again, suggesting that spatial differences extended to differences in mussel health. It is noted, that individuals of similar size were selected to reduce bias into the number of MPs observed as it was assumed that larger mussels accumulated more MPs. For this reason, the distribution of available mussel sizes is not equally represented.

It is noted that the T60-Powell River mussels which had significantly higher MP abundances were also significantly smaller in shell length compared to 7 of the 10 sites at T60 (p-values ranged <0.0001 – 0.005 for each comparison made). Corresponding to this, mean BCI for the T60 – PR mussels and these groups were not statistically different. This indicates that the mussels were smaller and of similar health to other mussels within the study, but contained a greater number of CSMP/GWW

It is important to consider that the results of this study detail the number of SMPs observed within the shell cavity of the mussel. At the time this experiment was started, methods had not yet been refined to investigate the number of SMPs within the digestive tract of individual mussels, while equally controlling for airborne contamination. Instead, values report the number of particles observed inside the shell cavity, inclusive of recently filtered (i.e. not yet digested), ingested and/or particles adhered to the gill surface or mantle (Kolandhasamy et al., 2018). The particles observed still have the

potential to accumulate within the mussel, however, given the relatively large size of particles observable (25µm – 5mm), translocation to tissues is not possible (Browne et al., 2008). For this reason, mussel accumulation was considered for the entire shell cavity, as it best reflects the potential physical mechanism of accumulation for the size range examined.

The number of MPs reported in this study fall within global values published within 2017 and 2018 for *M. edulis* (Catarino et al., 2017; Li et al., 2016; Qu et al., 2018), with fibres being the dominant particle type observed (Qu et al., 2018). At the time this report was written, only one other caged mussel study had been published. Catarino et al. (2018) housed blue mussels in areas along the coast of Scotland. Mean reported abundances for individuals were 3.0 ± 0.9 (SE) SMPs (for *M. edulis*). The authors then noted that visual microscopy (combined with fluorescence) resulted in false positive rate of 48 – 50%, lowering estimated abundances to 1.5 MPs/GWW. These values are similar to those reported in this study.

Considering the results of this study, it is concluded that MPs 25µm – 5mm did not accumulate within the shell cavity of the blue mussel (*Mytilus edulis*) over a 60-day period within the coastal British Columbia waters. There was only one observation of higher abundance of MPs between all sites and time periods. Although the T60 – Powell River mussels had significantly more plastics than all other sites at each time period, this is potentially due to the proximity to shellfish farming facilities and sewage outfalls (RDN, 2018).

Contrasting the recommendations of Catarino et al. (2017), researchers should consider the practicality of using blue mussels as indicators of MP pollution within the marine environment. Considering the low MP abundances within the shell cavity of mussels, and the difficulty in eliminating airborne contamination from both field sampling and laboratory analysis, it is not recommended that blue mussels be used to assess MP pollution. Considering only 17% of the SMPs observed were plastic, and then correcting these values for airborne contamination, MPs values equate to <1 particle/mussel. Comparing these numbers to values reported in British Columbia seawater (2,080 (± 2,190 SD) particles/m^3,(Desforges et al., 2014)), it is both more time efficient and cost effective to sample seawater directly, rather than utilizing the blue mussel to assess marine plastic pollution in local environments.

It is noted, however, that the small sample size and limited replication of this pilot study may have impacted observations of MPs accumulating within the blue mussel shell cavity. It is therefore suggested that the field program be repeated in similar locations with increased sampling over a longer duration of time to see if similar results persist. Cages could be placed in areas based on proximity to sewage and wastewater outfalls to determine if MP abundances change over time as a relation to source (Gies et al., 2018). This would also capture potential seasonal changes in MP abundances and differences in mussel filtration rates (Catarino et al., 2018; Jorgensen et al., 1990).

Furthermore, Shim et al. (2016) suggest that particles within the smallest size fraction of MPs (<25μm, the lower size limit of this study) likely increase exponentially in abundance within the marine environment. Microscopy equipment available at the time of this study was unable to resolve anything smaller than 25μm. It is therefore possible, that particles within this lower size fraction are present in higher amounts than reported within this study.

It too, is possible that particles reaching the lower limit of 1μm may translocate to tissues, and accumulate within the soft body of the blue mussel (Browne et al., 2008). Von Moos et al., (2012) found that high density polyethylene fluff (HDPE; 0 – 80μm size range; 2.5 g HDPE-fluff/L), translocated via endocytosis on the gill surface. Whereas the larger sized MPs within the study moved into the digestive system with some particles taken up by the lysosomal system. It is suggested that translocation has an upper size limit and is also species specific (Duis and Coors, 2016).

It is noted that during microscopy, fibres were observed inside the pores of some 20μm filter papers. In some instances, fibers were present both on top and underneath the filter paper being examined inside a plastic petri dish. This indicates that the 20μm pore size is a likely source of sample loss and potentially underestimated the number of fibres quantified per mussel. Mean fibre width was 20.4 μm (4.8 SE), suggesting that fibres may be lost depending on their orientation at the time of filtration. It is noted, however, that the number of fibres observed within the filter pores was low, suggesting sample loss was likely not a large source of error.

Considering the rigorous contamination control and use of enzymatic digestion, this study provides high resolution, representative results on the number of MPs found

within British Columbia blue mussels. Given the low numbers of MPs observed ranging in size from 25μm – 5mm, it is suggested that researchers attempting to quantify MP abundances in the marine environment focus on improving sampling techniques for seawater. Mussels should still be sampled, however, to determine abundances in relation to human health risk and potential for trophic transfer within the food web (Guzzetti et al., 2018). Technology capable of observing MPs <25μm is crucial in truly understanding MP abundances within the blue mussel, as the possibility of accumulation within the soft tissues of individuals is greatest within this size fraction.

Chapter 3.

Microplastic rejection, elimination and retention in blue mussels (*Mytilus edulis*)

3.1. Introduction

To date, microplastic (MP) exposure experiments involving the blue mussel (*Mytilus edulis*) primarily involved one particle type at highly elevated concentrations (Browne et al., 2008; Cole et al., 2016; Farrell and Nelson, 2013; Kolandhasamy et al., 2018). Only in the past year have studies began utilizing MPs of varying polymer types in concentrations that are considered relatively environmentally relevant (Kolandhasamy et al., 2018; Qu et al., 2018) . Furthering this, most studies have examined the number of particles retained within the mussel itself. At the time this experiment was conducted, studies looking at the proportion of plastics rejected as pseudofaeces (PF) and eliminated as faeces (F) had not been published in the scientific literature (to the best of the authors knowledge). Hence, the purpose of this study is to examine particle fate post mussel filtration, to better understand the physical mechanism particles undergo within the shell cavity of the blue mussel.

Blue mussels feed by filtering particulate matter from the water column. Microalgae ranging in size from 2 – 40 µm is the preferred food source (Pales Espinosa et al., 2016) for *Mytilus edulis* individuals. Feeding is nonselective, hence all particulate matter present in the water column (referred to as seston) is brought into the shell cavity, including detritus, sediment and organic matter. Seston is brought into the mantle cavity through the incurrent siphon, filtered over the gill surface and moved toward the labial palps for sorting (Bayne et al., 1993; Browne et al., 2008). It is generally agreed that high quality particles are brought to the mouth for ingestion and low quality particles are directed to the excurrent siphon for rejection (Bayne et al., 1987; Pales Espinosa et al., 2016; Widdows et al., 1979). The rejected material is referred to as pseudofaeces (PF) and eliminated material (post digestion) is referred to as faeces (F).

It is reported that the northeastern Pacific Ocean has a mean abundance 2,080 (± 2,190 SD) MPs/ m^3. Of the 34 stations sampled, a coastal location exhibited the

greatest abundance of MPs at 9,180 particles/m^3 (4 – 27 times greater than offshore sites, (Desforges et al., 2014)). The majority of particles recorded in seawater were fibres (~70% for coastal sites) a trend that is exhibited globally (Carr, 2017; Duis and Coors, 2016; Lusher et al., 2015; Wang et al., 2017) and extends to the dominant particle type reported in mussels (Amaral-Zettler et al., 2016; De Witte et al., 2014; Li et al., 2016; Lusher et al., 2017; Rochman et al., 2015). This phenomenon is often attributed to the high number of fibres released into the marine environment through waste water effluent (Gies et al., 2018). Hence, the particle types most often quantified are fibres, fragments, foams, films, and spheres (also known as beads or pellets (Hidalgo-Ruz et al., 2012; Lusher et al., 2016; Marine & Environmental Research Institute, 2015)).

It is currently assumed that MPs accumulate within mussel body organs or through adherence (Browne et al., 2008; Kolandhasamy et al., 2018). Considering the low numbers reported within the shell cavities of mussels (i.e. plastics that may be present in the digestive tract, adhered to the gill surface and/or mantle at the time of extraction), however, it appears that individuals are capable of either rejecting MPs at the source (as PF) or eliminating them post digestion (as F). It is likely that a proportion of MPs available in the surrounding marine environment are observed within the shell cavity of the mussel, providing a snap shot of exposure at the time of collection. To date, no study has quantified the number of particles eliminated in the faeces, rejected within the pseudofaeces or retained within the shell cavity of the mussel (particles present within the shell cavity includes the pallial fluid, material present in the digestive tract and/or particles adhered to the soft tissues and mantle) after an acute exposure event (as of February, 2018 when the study design was completed).

Using environmentally relevant concentrations of MPs, blue mussels were acutely exposed to MPs to assess particle fate after mussel filtration. Exposure occurred over a 24-hour period to ensure the digestion process was completed. A combination of polymer types and shapes at environmentally relevant concentrations were used to mimic potential future concentrations found in British Columbia seawater, a factor that has been considered a data gap in recent exposure experiments (Amaral-Zettler et al., 2016; Green et al., 2017; Lusher et al., 2016; Phuong et al., 2017; Qu et al., 2018). It is assumed that MPs will be observed within the PF and F and a proportion of the particles exposed will remain within the shell cavity of the mussels at the end of the 24-hour

exposure period. Results from this study will further our understanding on the use of the blue mussel as an indicator of MP pollution within the marine environment by better understanding particle fate post filtration.

3.2. Materials and Methods

Study Design

MPs within the marine environment are generally characterized as fibers, fragments and spheres (aka beads or pellets). Hence, the objective of the controlled feeding experiment was to 1) expose blue mussels (*Mytilus edulis*) to environmentally relevant concentrations of MPs that varied in particle shape and 2) determine differences in rejection, elimination and retention of the three types of particles over a 24-hour period. At the time of the study design, no experiments using environmentally relevant, variously-shaped MPs had been published. Environmentally relevant concentrations were determined through a literature review based on the abundance and composition of polymer shapes found in open waters within the marine environment (Desforges et al., 2014; Gago et al., 2018; Zhao et al., 2014)

Table 3.1 summarizes results from the study used to set exposure concentrations for the feeding experiment (Desforges et al., 2014). Completed in the nearshore waters of British Columbia, values from this study were converted to the number of MPs per litre of seawater for three potential future concentration groups. Values were doubled to represent conservative, 10 year increases in marine pollution. This is considered a conservative estimate as it is assumed marine pollution input will increase by an order of magnitude from 2015 – 2025 and will not peak until 2100 (Jambeck et al., 2015).

The three concentration groups therefore represent potential MP abundances within the Strait of Georgia (SOG) in 30, 40 and 50 years (hereafter referred to as low, medium and high concentration groups, respectively). Composition of particle shapes were divided to reflect values reported by Desforges et al. (2014); 70% of the particles were fibres and 30% classified as 'other'. For the purposes of this study, the 'other' particles were further categorized as 10% pink polyethylene terephthalate (PET) spheres, 10% PET blue spheres and 10% acrylonitrile butadiene styrene (ABS) fragments.

Microplastic Sample Preparation

MPs of three particle shapes were used in the experiment, polyester (PE) fibres, PET spheres and ABS fragments in three concentrations Table 3.1. MPs were prepared for each concentration group; low (73 MPs/L), medium (148 MPs/L) and high (296 MPs/L), representing potential 30, 40 and 50-year MP abundances, respectively. Values were based on highest reported MP contamination levels reported for the SOG, BC (9,180 MPs/m^3; Desforges et al. (2015)). Values were doubled to represent potential increases in plastic pollution. This is considered conservative based on marine pollution input trends suggested by Jambeck et al. (2015). Potential future values from 30 – 50 years were chosen as it is assumed this is when the signal to noise ratio for working within 1L of water would be large enough to observe a response.

Small fibers were created by shedding fibres from a piece of red, PE fabric and cutting them to shorter lengths. Fibres were examined under dissecting scope, individually picked and placed into a glass vial partially filled with filtered tap water. Fresh water was used as vial preparation occurred weeks before the experiment and biological material within seawater may have died and decayed before trials began. This process was repeated until the appropriate number of fibres were collected for the designated concentration. In total 15 vials of fibres were created for the experiment (5 each for low, medium and high concentration groups).

Fibre lengths were determined after the experiment was completed. A subset of fibres (20%, randomly selected) were measured under the dissecting microscope using ImageJ for each of the samples processed (i.e. fibres present in the PF, F, 7.5 L fish bowl, 1 L beaker and mussels examined at each sample concentrations). Fibre lengths ranged from 129 µm – 5 mm with a mean length of 890 µm (30.6 SE). Mean fibre width was 16.3 µm (0.09 SE).

MP spheres were purchased from Cospheric LLC in two size ranges and colours to represent size fractions above the lower limit of detection for the study (lower limit = 25 µm based on microscope resolution). Pink, fluorescent, PET size = 45 – 53 µm; density = 1.09 g/cm3 and blue PET spheres size = 90 – 106 µm; density = 1.08g/cm3. Spheres were hydrophobic and remained within the seawater surface layer during trials. PET spheres were placed for an hour in a petri dish with 1 mL of a biocompatible

surfactant solution 0.1% v/v (Tween® 20, Cas 9005-64-5, Sigma-Aldrich) to create a hydrophilic surface, causing the particles to sink. The required number of MP spheres for each treatment, determined under microscope, were placed in vial cap, photographed and pipetted (glass pipette with rubber bulb) into a vial containing filtered water and microfibers. The lid was examined under the dissecting microscope to ensure all particles entered the vial.

Microfragments were created by shaving a black ABS pipe. Shavings were placed inside a plastic vial with a metal bead and shaken 25 times/s for 2 minutes (Mixer Mill MM 400). Fragments were counted using the method describe for spheres.

Mussel Acclimatisation

The same stock of blue mussels used in the caged mussel study (see Chapter 2) were used in the feeding experiment. Live mussels were received from Powell River, BC (Taylor Shellfish Farms) in January 2017. Individuals were housed in a lantern net within a settling tank at the Vancouver Aquarium until experimental trials began. Raw seawater is pumped directly from Burrard Inlet, entering the settling tank before filtration. Mussels were removed from the lantern nets and transferred to the Pacific Science Enterprise Centre in West Vancouver, BC in January, 2018. Individuals were placed into two holding tanks receiving seawater pumped directly from Howe Sound, BC. Only sediment is removed in the filtration process and ambient biological material remains present.

Individuals acclimatized to laboratory conditions for two months prior to the start of experimental trials with a 12-hour photoperiod. Mussels were fed weekly using Instant Algal Paste (IAP; Innovative Aquaculture Products LTD) composed of naked flagellates ranging in size from 2 – 5 µm (*Tahitian isochrysis*, *Isochrysis galbana*, *Pavlova lutheri*, and *Nannochloropsis oculata*; concentration = ~25 billion cells/mL*).* Mussels were fed 1 mL of paste diluted in 200 mL of seawater. Algae was added and water turned off for 1.5 hours for feeding.

Experimentation Overview

Precautions were taken to avoid MP contamination. Equipment was rinsed inside laminar flow hood 3 times with filtered water and stored in tinfoil. Filtered seawater (1 µm

glass FP) was used for rinsing mussels and experimental seawater was sieved (63 µm). Laboratory technicians wore 100% cotton lab coats and nitrile gloves.

Each trial was repeated 5 times with a control, low, medium and high concentration group (n = 20). The control group underwent the same experimental procedure as the low, medium and high concentration groups, however, only algae (i.e. no MPs) was added to the test tanks. Before the start of each observation period, twelve individuals were randomly selected from the holding tanks and placed into 8L of sieved seawater (63 µm) with airflow. This reduced food availability and promoted feeding the following day.

Mussels were exposed to MPs in 1 L of filtered seawater (63 µm sieve) for a 5-hour period (Table 3.1). Individuals were monitored for PF (representing MP rejection) and F production (representing MP elimination) in real time. Due to the intensive observation period and real-time sample collection process, 5-hours was the maximum observation period possible. Mussels were moved to a 7.5 L fish bowl for a further 17-hours to continue the digestive process and killed 24-hours after the initial exposure event. MPs extracted from the mussels were used to determine MP retention.

Experimentation Process

On the morning of each trial, mussel shells were cleaned and byssal threads gently cut off shell exterior. Mussels were rinsed and placed into a labelled 1 L glass beaker, with rinsed stir bar, and 950 mL filtered seawater. Three mussels were placed in each beaker on magnetic stir plate (130 rpm, Super-Nuova SP135935). Mussels were gently moved to one side of the beaker, opposite the metal stir bar. Beakers were covered with tinfoil to reduce airborne contamination (Figure 3.1a). Water quality measurements were taken in the 1 L beaker to ensure WQ parameters were within normal environmental range before starting the experiment (temperature, salinity, pH and dissolved oxygen).

MP vial contents were tipped into each beaker and rinsed with filtered water to remove contents. The volume of filtered seawater was brought to 1 L in each beaker. Vials and lids were checked for remaining pink spheres using fluorescence. If spheres were observed on gloves or pipetting equipment, particles were recorded and counted

as "lost". Stir bars created water movement and ensured MPs did not sink to the bottom of the beaker. Pink microspheres were observed floating throughout the 5-hour exposure period (checked periodically after PF/F collection). Beakers were equipped with airflow to provide a steady air supply and further aid in MP circulation. Only pink spheres fluoresced, so it was not possible to check for sample loss for any other particle types (fibres, fragments or blue spheres). Due to the small size of the pink spheres, it is assumed sample loss for the other 3 particles shapes is lower or equal to those reported for the pink spheres.

Mussels were monitored for PF and F production roughly every hour. PF (appearing white and fluffy with irregular edges) and F (appearing brown and pellet like with rounded edges) were collected via pipette, and placed into 20 mL vials. One vial was assigned for each concentration group (control, low, medium and high) and sample type (PF or F). Samples for all three mussels were pooled into the same vial to avoid pseudoreplication.

After 5 hours of feeding, mussels were rinsed and placed into a 7.5 L fish bowl (one each for control, low, medium and high groups) containing freshly filtered seawater for depuration overnight. Airlines were rinsed and transferred to the fish bowls to provide airflow. Water quality measurements (salinity, pH, temperature, dissolved oxygen and ammonia) were taken in each 7.5 L fish bowl before mussels were transferred to determine pre-treatment conditions. It is noted that WQ parameters were not measured in the 1 L beaker after mussels were removed due to a high risk of sample loss and/or contamination from equipment probes. 1 L beaker contents were required for filtration to determine the number of MPs that were not filtered by mussels. For this reason, post-treatment WQ was not collected in the 1 L beakers.

Fish bowls were examined for PF and F production the following day, roughly 24-hours post MP exposure. PFs and Fs were collected into the same vials from the previous day. Samples were refrigerated (4°C) for processing. Mussels were removed and rinsed. One mussel from each beaker was frozen for MP analysis (-20°C) and two were processed for health effects. Health effects were analysed by Gabriela Aguirre and are not being presented in this report. Water quality was again measured and recorded to determine post-treatment conditions.

Microplastic Extraction and Enumeration

All volumes of water exposed to MPs (1 L beakers and 7.5 L fish bowls) were immediately vacuum filtered onto a 20 µm PC filter paper for MP enumeration. PF and F vials were digested using the Corolase 7090 enzyme (1 mL) on a hotplate (55 – 60°C; 200rpm) overnight (~20 hours) to breakdown biological material. The digestate for each sample was filtered onto a 20 µm PC filter paper and placed into a plastic petri dish with lid to dry. Mussels were weighed prior to dissection (still in the shell) to determine total body wet weight (TWW) and after shell removal to obtain body wet weight (BWW). Procedural blanks were not required as only plastic particles added (pink fibres, pink and blue spheres and black fragments) were quantified. Observed contamination particles were ignored during microscopy.

Filter papers were examined using visual microscopy (Olympus SZX16 microscope (up to 18.4X magnification) with Olympus DP22 camera and DP2-SAL software, Olympus Corporation, Tokyo, Japan). Samples (PF, F, 7.5 L fish bowl, mussel and 1 L beaker) were examined on a coloured grid and the number of fibres, fragments, pink and blue spheres recorded.

Data Analysis

A single-factor randomized complete block analysis of variance (SF RCB ANOVA) was completed to determine if mean water quality changed significantly from mussel placement into the 7.5 L fish bowls to their removal (both time and concentration were fixed factors). Because WQ was not measured in the 1 L beaker post MP exposure (to avoid contamination and or sample loss from equipment probes), WQ measurements within the 1 L beaker were not possible.

Body condition index (BCI) was calculated by comparing BWW to TWW (Bråte et al., 2018; Brooks et al., 2015). Mean BCI was calculated for each concentration group (n = 5) and a SF RCB ANOVA was performed to determine if mean BCI differed between groups. Mean BCI, BWW and TWW were also calculated.

$$\boldsymbol{BCI = \frac{body\ wet\ weight}{total\ wet\ weight} \times 100}$$

It is assumed that particles remaining in the 1 L beaker were not filtered by the mussels during the 5-hour exposure period. Thus, the number of particles filtered by mussels was calculated by removing the values reported for each 1 L beaker from the total number of MPs added for each concentration group. Proportions were analyzed separately for each particle shape at all concentration groups. Fragments were not included in the data analysis due to low recovery rates.

$$\boldsymbol{Particles\ Filtered = Total\ MPs - MPs\ Observed\ in\ 1L\ Beaker}$$

The mean proportion of MPs recovered in the PF, F, 7.5 L fish bowl and mussel was determined by comparing the number observed in a sample to the number of particles filtered for that concentration group. All values are reported as a percentage. A SF RCB ANOVA was completed to determine if the mean proportion of MPs observed within a sample (PF, F, 7.5 L fish bowl, or mussel) differed between concentration groups. The same procedure was repeated to compare the mean proportion of fibres, pink spheres and blue spheres individually. It is noted that the number of plastics observed in each mussel were tripled to account for the two individuals sent for health analysis. For this reason, proportions of MPs retained by the mussel are >100%.

A Tukey-Kramer multiple comparison test was used to look for differences in the proportion of particle types observed in each of the samples when a significant difference was observed. All analysis was completed in JMP (JMP, 13.0.0).

Table 3.1 **Microplastic (MP) concentrations for low, medium and high treatment groups were based on values reported in Desforges et al. (2014). Concentrations of MPs for potential 30, 40 and 50 year concentrations are referred to as low, medium and high treatment groups, respectively. These concentrations are based on current values and projected future trends (Jambeck et al., 2015). Particle abundances were subdivided to reflect particle shapes present in the Strait of Georgia, British Columbia waters (70% fibres, 30% other). Mussels were exposed to MPs at each concentration to determine particle fate post filtration. That is the proportion of plastic observed in the pseudofaeces (rejected plastics), faeces (eliminated plastics) and mussel (retained plastics) was quantified and compared.**

Scenario	Experimental Concentration Name	Total MPs (m^3)	Total MPs (L)	Fibres (n)	Pink Spheres (n)	Blue Spheres (n)	Fragments (n)
Strait of Georgia in 2014*	NA	9,180	9	0	0	0	0
Potential 30 Concentration	Low	73,440	72	51	7	7	7
Potential 40 Concentration	Medium	146,880	148	103	15	15	15
Potential 50 Concentration	High	293,760	296	206	30	30	30

Note** Strait of Georgia concentrations from (Desforges et al., 2014)

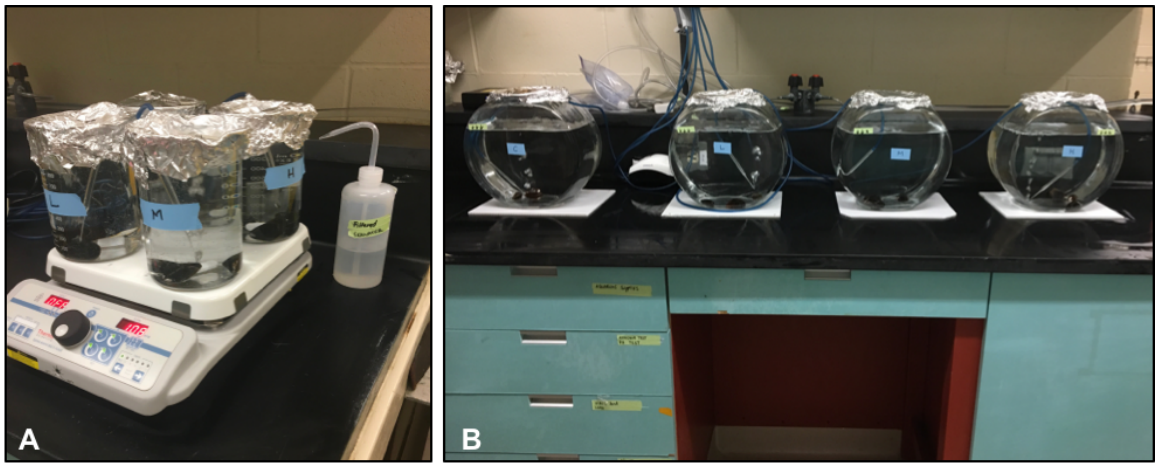

Figure 3.1 **Mussels were exposed to microlastics (MPs) at three concentrations in 1 L beakers on metal stir-plate for a 5-hour period (photo A). A stir bar (130rpm) and air flow ensured particles continued to circulate throughout filter feeding. Beakers were labelled as control (C), low (L), medium (M) and high (H) concentration groups. Beaker position was randomized on the hot plate for each trial. Mussels were subsequently moved to a 7.5 L fish bowl (photo B) for overnight depuration. Both tank set ups included continuous airflow. All openings were covered with tinfoil to reduce risk of airborne contamination.**

3.3. Results

Body Condition Index & Water Quality Parameters

Mean BCI ranged from 30.0 (3.1 SE) to 35.8 (2.3 SE) and did not differ between concentration groups (F-statistic = 0.70, p-value= 0.56). This indicates that mussels used in the experiment were of similar health (Figure 2.6).

Mean values for WQ parameters are provided in Table 3.2 for 1 L beaker and 7.5 L fish bowl. Of the 5 parameters measured, no significant differences were observed in mean salinity (p-value = 0.87) or ammonia (p-value = 0.16) from the start to end of the experiment.

A two-factor RCB ANOVA (time and concentration = fixed effects) determined that mean temperature increased significantly within the 7.5 L fish bowls (p-value < 0.0001) overnight. Mean temperatures for the four concentrations groups at the start of exposure ranged from 10.3°C (0.1 SE) – 11.4°C (0.5 SE) and increased to 17.4°C (0.1) – 17.7°C (0.1 SE) at the end of exposure. No difference in mean temperature between concentration groups was observed (p-value = 0.98), indicating that the increase in mean temperature was a by-product of the closed tank system being located in a heated room.

Similar to temperature, mean pH increased overnight (p-value < 0.0001) from pH -7.9 (0.1 SE) to 8.2 (0.0 SE) – 8.3 (0.1 SE) for the fish bowls. Mean dissolved oxygen ranged between 12.5 ppm (0.2 SE) – 13.2 ppm (0.2 SE) when mussels were placed in the fish bowl, and decreased to 9.0ppm (0.3 SE) – 9.5ppm (0.3 SE) which was significant (p-value < 0.001). Similar to temperature, differences in pH and dissolved oxygen were not significantly different between concentration groups (p-value = 0.99 and 0.95, respectively), indicating that the changes were unlikely to affect mussel behaviour (as all concentration groups, inclusive of the control, exhibited the same trends).

Sample Loss & Contamination

A loss of 7 pink spheres was recorded during experimentation (2.7% of the 260 added). Only pink spheres fluoresced, so it was not possible to check sample loss for any other particle types. Of the 2,580 MPs introduced in the study (fibres, fragments and

spheres), 2.3 % (n = 61) were recovered in the control samples, indicating that cross contamination was also a low source of error.

As a wet lab with clean room conditions was not available for this study, 100% elimination of airborne MP contamination was not possible. It is noted that once reduced to the 'micro' size range, black ABS fragments became transparent and grey in colour making them difficult to identify among background contamination. Hence, fragment recovery rates were lowest (25%) compared to fibres (108%), pink (68%) and blue spheres (56%). For these reasons, fragments were eliminated from data analysis. Note that recovery rates are calculated using tripled mussel values to account for individuals sent for health analysis. For this reason, the number of fibres recovered is >100% as exact values of MPs retained within mussels is unknown.

Microplastics Filtered by Blue Mussels

MPs were observed in all samples examined (PF, F, mussel, 7.5 L fish bowl and 1 L beaker filter papers). Table 3.3 details the proportion of MPs recovered in the PF, F, mussel and 7.5 L fish bowls. No outliers were observed in any of the samples enumerated (Figure B1).

The mean proportion of filtered MPs was lowest in the PF (4%, 2% and 3% for the low, medium and high concentrations, respectively) and highest in the mussel (57%, 68%, 46% for low, medium and high concentrations, respectively). MPs contained within the 7.5 L fish bowl water were not decipherable as PF or F and therefore treated independently. The SF RCB ANOVA revealed that the mean proportion of plastics in the mussels (for all particle types) was significantly higher compared to proportions recovered in the PF, F and 7.5 L beaker (p-value = <0.001). Trials utilizing extremely high concentrations of algae confirmed that mussel digestion occurred within hours. After IAP was added, mussel faeces expelled were bright green, indicating digestion had occurred (whereas shortly before faecal pellets were a dull brown). Algae concentrations used in the experiment were therefore lowered significantly to ensure high particle count (total count of algae and MPs) did not affect mussel filtration behaviour.

When considering particle shapes individually, there were significantly more fibres in mussels from the medium (99%) and high (86%) concentration groups (p-value <0.001) compared to mean proportion of fibres in mussels from the low (57%). This difference was also significant when comparing mean proportion of fibres in the PF, F and 7.5 L fish bowl samples (mean proportions ranged from 5% - 15% for all concentrations; see Table 3.3).

Mean proportion of pink spheres observed in PF, F, mussel and 7.5 L fish bowl did not differ between concentration groups (p-value = 0.03). The same trend was exhibited for blue spheres (p-value = 0.45). Figure 3.4 illustrates, however, blue spheres were absent from PF samples at all concentrations (0%). Pink spheres were only observed in PF for the low (4%) and high (1%) concentration groups, while fibres were recovered in all four sample types (PF, F, 7.5 L fish bowl and mussels).

Table 3.2 **Water quality was measured at the start of each experiment. Mean temperature (°C), salinity (ppt), dissolved oxygen (mg/L), pH and ammonia (ppm) for 5 experimental feeding trials at 3 microplastic (MP) exposure concentrations (control, low, medium and high) are provided below. Measurements taken in the 1 L beaker and 7.5 L fish bowl were measured before mussels were placed inside the tanks. Parameters were measured again within the 7.5 L beaker the following day. 1 L beaker measurements were not taken post-exposure to avoid MP contamination and/or sample loss from measuring probes.**

Concentration	Tank Size / Time Measurement Taken	Temperature (°C)		Salinity (ppt)		Dissolved O_2 (mg/L)		pH		Ammonia (ppm)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Control	1 L beaker / Before	12.56	0.88	27.70	1.20	11.42	0.48	7.96	0.10	0.00	0.00
	7.5 L Fish Bowl / Before	10.28	0.30	28.56	1.17	12.92	1.31	7.89	0.05	0.00	0.00
	7.5 L Fish Bowl / After	17.58	0.13	28.52	1.03	9.29	0.83	8.25	0.02	0.00	0.00
Low	1 L beaker / Before	12.28	0.92	28.58	0.41	11.67	0.79	7.96	0.08	0.00	0.00
	7.5 L Fish Bowl / Before	10.34	0.31	28.28	1.00	13.13	0.78	7.90	0.05	0.00	0.00
	7.5 L Fish Bowl / After	17.42	0.08	28.32	0.94	9.52	0.61	8.26	0.04	0.00	0.00
Medium	1 L beaker / Before	12.50	0.76	27.88	1.11	11.45	0.26	7.99	0.08	0.00	0.00
	7.5 L Fish Bowl / Before	10.48	0.36	28.32	1.06	13.18	0.54	7.91	0.04	0.00	0.00
	7.5 L Fish Bowl / After	17.42	0.13	28.50	1.03	9.12	0.56	8.26	0.04	0.00	0.01
High	1 L beaker / Before	12.32	0.75	27.92	0.88	11.48	0.46	7.98	0.07	0.00	0.00
	7.5 L Fish Bowl / Before	11.36	1.12	28.40	0.79	12.49	0.48	7.91	0.02	0.00	0.00
	7.5 L Fish Bowl / After	17.72	0.23	28.52	0.92	9.04	0.63	8.24	0.05	0.00	0.00

Table 3.3 **Data describes the mean proportion of fibres, pink spheres, blue spheres and total MPs observed in the pseudofaeces (PF), faeces (F), 7.5L fish bowl (where plastics could not be determined to be from PF or F) and within the mussel shell cavity. Because only one mussel was examined for MPs, the number of plastics observed was tripled to account for the missing two mussels sent for health anaylsis (not reported within this study). Therefore, values for mussels do not add to 100% due to the associated data adjustment. Percentages were calculated based on the total MPs filtered (Total MPs Added – MPs Observed in 1L Beaker). The column 'Total MPs' describes the percentage of MPs observed in each sample for all particle types. A higher proportion of MPs were observed in mussels than the PF, F or the 7.5 L fish bowl ($p < 0.001$).**

Concentration	Sample	N	Fibres	Pink Spheres	Blue Spheres	Total MPs
			Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Low	Pseudofaeces	5	8 ± 7%	4 ± 9%	0 ± 0%	4 ± 7%
	Faeces	5	8 ± 4%	0 ± 0%	0 ± 0%	3 ± 5%
	7.5 L Fish Bowl	5	13 ± 7%	8 ± 18%	0 ± 0%	7 ± 12%
	Mussel	**5**	**58 ± 52%**	**94 ± 137%**	**19 ± 26%**	**57 ± 86%**
Medium	Pseudofaeces	5	5 ± 5%	0 ± 0%	0 ± 0%	2 ± 4%
	Faeces	5	14 ± 13%	3 ± 6%	3 ± 7%	7 ± 10%
	7.5 L Fish Bowl	5	15 ± 17%	9 ± 13%	10 ± 10%	12 ± 13%
	Mussel	**5**	**99 ± 78%**	**67 ± 79%**	**37 ± 41%**	**68 ± 69%**
High	Pseudofaeces	5	6 ± 8%	1 ± 2%	0 ± 0%	2 ± 5%
	Faeces	5	13 ± 7%	10 ± 10%	1 ± 2%	8 ± 8%
	7.5 L Fish Bowl	5	9 ± 5%	10 ± 12%	4 ± 6%	8 ± 8%
	Mussel	**5**	**86 ± 59%**	**23 ± 35%**	**28 ± 30%**	**46 ± 50%**

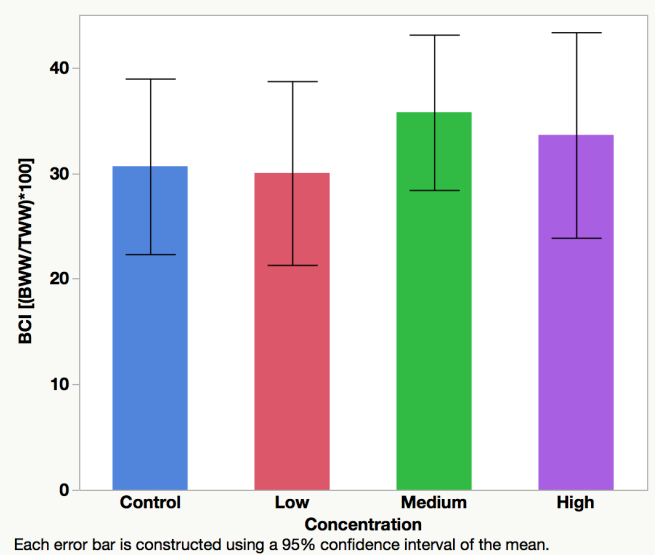

Figure 3.2 **Mean body condition index (BCI) for mussels (n = 15 / treatment group) exposed to microplastics (MPs) at low, medium and high concentrations. Mean BCI did not differ across groups, indicating that mussel health was similar throughout the experiment (p = 0.56).**

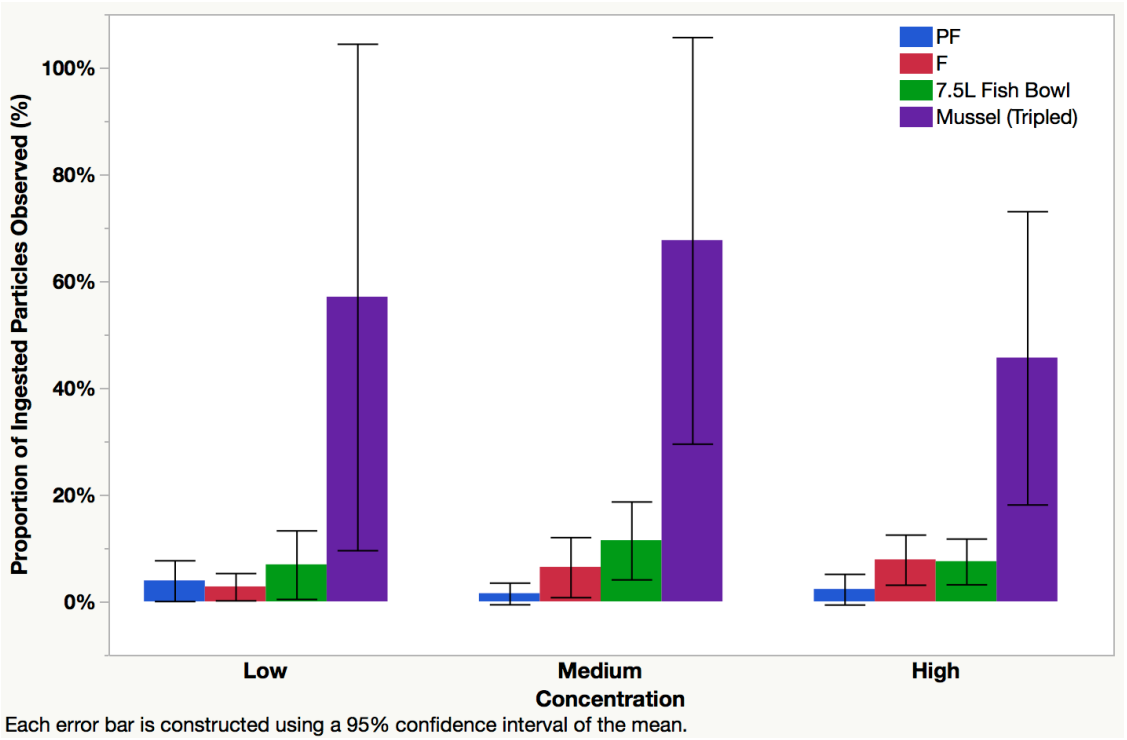

Figure 3.3 **Mussels retained significantly more microplastics (MPs) than was observed in the pseudofaecse (PF), faeces (F) or 7.5L fish bowls for all concentration groups (p-value = <0.001). Note that only one of three mussels was examined for MPs. To account for missing mussels, mussel values were tripled. Hence, proportions exceed 100%. MPs observed within the 7.5 L fish bowl samples indicate that particles were either rejected as PF or eliminated as F overnight. Particles in this sample were unable to be identified as either PF or F and were pooled.**

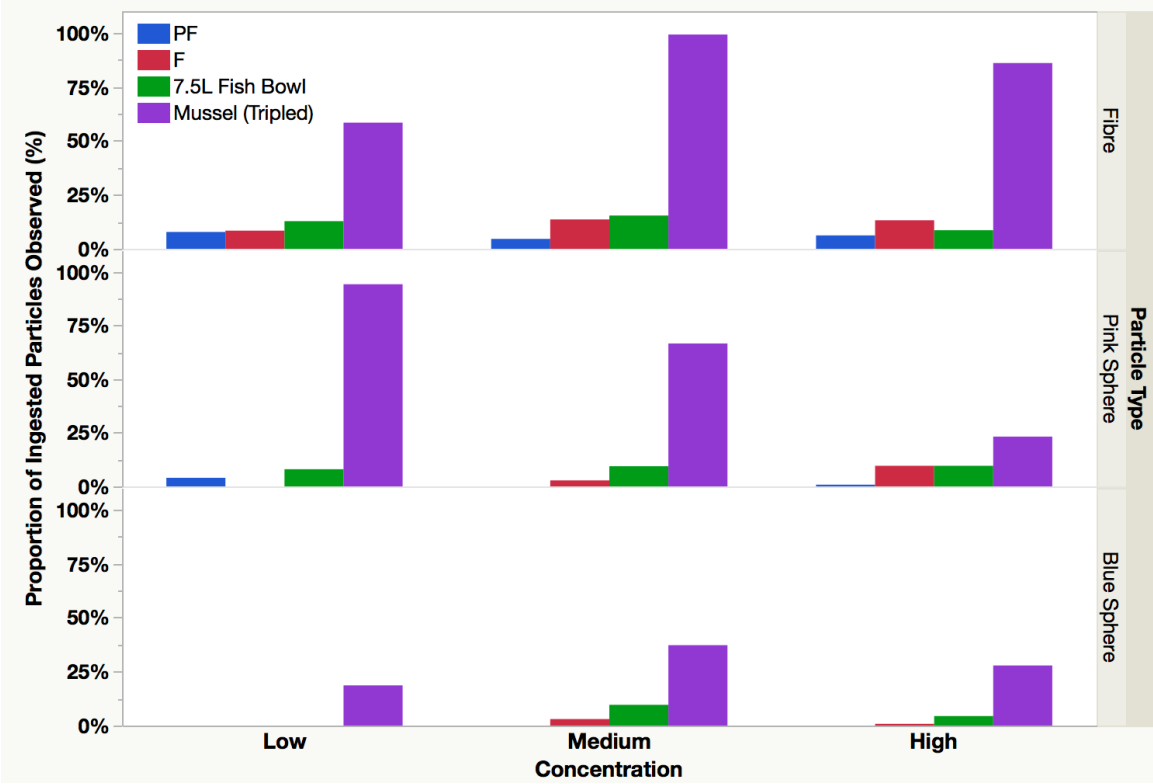

Figure 3.4 **Bar graph showing the proportion of each microplastic type recovered (fibres, pink and blue spheres) within the pseudofaeces (PF), faeces (F), 7.5 L fish bowl and mussel samples for all concentrations groups (low, medium and high). Fibres were observed in all sample types. Pink and blue spheres were absent from the PF and/or F samples at low, medium and high concentrations. Signficantly more MPs were observed in the mussel than the PF, F or 7.5 L fish bowl samples (p-value = <0.001).**

3.4. Discussion

This study confirms that MPs are rejected in PF, eliminated as F and retained within the mussel shell cavity after filter feeding occurs. Overall trends indicate that MPs filtered within five hours of exposure are most prominently retained within the mussel shell cavity 24-hours after an acute exposure event. Based on preliminary trials, it was observed that the mussels in this experiment ingested particles and passed them through the digestive tract within hours of exposure (as faeces turned bright green), suggesting a 24-hour exposure regime was enough time to witness particles passing through the blue mussel.

Fibres were the most prominently retained particle type, mimicking results presented in the CMS (see Chapter 2). The proportions of particle types ingested, however, reflect the numbers added at each concentration. More fibres were retained within the mussel as it was the dominant particle type (added at 70% of the total), which was expected. It is noted, however, that fibres were observed in all samples examined, whereas blue spheres were not observed in the PF at low and medium concentrations. Similarly, pink spheres were absent in the F at low concentrations and absent from the PF at medium concentrations. These results suggest that there may be a preference to retain the smaller sphere shaped particles over the larger fibres as this is closest to the preferred size range of phytoplankton ingested by the blue mussel, however, further experimentation is needed to better understand the implications of these observations.

The highest number of particles rejected and/or eliminated were observed within the 7.5 L fish bowl, collected a full day post exposure. This suggests that mussels were still digesting MPs 5-hours after the exposure event and continued to reject/eliminate MPs overnight. It is likely that the 24-hour trial period was too short to fully understand particle fate after an acute exposure event. Preliminary trials suggest that the mussel digestion process occurs within hours (as green faecal pellets were observed ~1hr post exposure to highly elevated algal concentrations), indicating that the 24-hour exposure period was sufficient to observe MPs within mussel faeces.

It is interesting to note that virgin plastics (i.e. plastics that are not weathered) were used in this study to determine the fate of MPs after filter feeding. Research suggests that weathered plastics mimic food particles more so than virgin plastics as

uptake rates of PET fragments is significantly higher in blue mussels compared to virgin plastics when measured by weight (Bråte et al., 2018). Moving forward, it is suggested that weathered MPs be used when completing laboratory experiments focused on MP fate post filtration.

It is possible that a greater number of particles may be ingested if weathered plastics are used. Particular attention should be made as to the number of particles found in the faeces, post digestion. If more particles are ingested, it is possible a great number of particles will be observed within the faeces as the caged mussel study suggests physical accumulation of MPs (25µm – 5mm) does not occur within the blue mussel. Alternatively, if the burden to process the particles becomes too great at the elevated future concentrations, physical accumulation of MPs within the shell cavity may be possible. Establishing this 'tipping point' is important to better understand the risk of detrimental effects to the blue mussel.

Considering these challenges, it is suggested that the experiment be repeated using weathered MPs at similar concentrations. However, trial length should be extended to determine MP fate following chronic exposure events. Browne et al. (2008) determined that mussels exposed to high concentrations of MPs did not exhibit a decline in the number of particles present within the shell cavity until 12-days post exposure. It is noted, however, that MPs in this study were within the preferential size range blue mussels feed on phytoplankton (3.0µm & 9.6µm). At this size fraction, the mussels ingested particles and translocation to tissues was observed, indicating a physical accumulation of MPs within the soft tissue of the animal. Browne et al. (2008) suggested that these smaller pieces are likely to accumulate more readily than larger plastics such as the ones used in this exposure experiment.

Exposing mussels to MPs in intervals would better mimic exposure within the marine environment. Focusing on plastics within the preferential size range of mussel feeding (4 – 20µm (Pales Espinosa et al., 2016)) will better represent mussel behaviour within the marine environment. Equipment is needed to both visually identify and enumerate MPs at this size fraction. If possible, equipment capable of observing particles within the soft tissue of the animal could help understand the physical mechanism of accumulation. Utilizing smaller, environmental concentrations, however, is instrumental in understanding risk.

Currently, research has shown the deleterious effects of MP ingestion exists, however, risk to health relative to other marine contaminants is poorly understood (Smith et al., 2018). Researchers need to better understand the residence time of MPs in the shell cavity of blue mussels and the rate of retention at differing size fractions and concentrations. This information can in turn be used to inform the aquaculture industry to provide parameters on depuration within filtered seawater and the potential for trophic transfer of MPs within the marine food web.

Chapter 4.

Discussion

It is important to look at microplastics (MPs) in blue mussels to establish baseline abundances for future research. At the start of this study, very little literature existed on the number of MPs in British Columbia blue mussels (*Mytilus edulis*). The results of the caged mussel study provided a novel way to quantify accumulation and assess the practicality of using the blue mussel as an indicator of MP pollution for particles ranging in size from 25µm – 5mm. Although the results indicate that blue mussels do not accumulate MPs (in this size fraction) in a similar manner to PCB's, PAH's, metals and petroleum, it is important to realize that blue mussels and other shellfish are being continually exposed to anthropogenic debris that is of low nutritional value. This likely incurs an energetic cost that may affect physical and reproductive health. As plastic pollution is theorized to continue to increase, it is important to continue studying MP transport and fate to better understand the risk this contaminant poses on our marine ecosystems.

A 60-day field experiment was conducted to determine if MP accumulation (for particles ranging in size from 25µm – 5mm) could be quantified in the blue mussel (*Mytilus edulis*). After standardizing for contamination (1.26 MPs/PB), mean MP abundance equated to 0.43 (0.06 SE) CSMP/GWW. Furthering this, 17% (n = 11) of the 66 SMPs sent to FTIR were confirmed plastic, indicating that most anthropogenic particles were synthetic, but not MPs. Inter-site comparisons of MP abundances were not significant for the three sampling periods (with one exception, T60 – Powell River mussels had more SMPs). Rigorous contamination control and polymer identification confirmed that previously reported results within the blue mussel likely overestimated true plastic loads.

Complimenting this, a feeding experiment utilizing environmentally relevant, multi-shaped MPs was completed to determine particle fate post filtration. Mussels were observed in PF and F production to determine the proportion of plastics in each. Mussel tissue was also digested (using enzymes) to determine the proportion of particles remaining within the shell cavity 24-hours post exposure. Fibres were the most

prominently retained particle type, with significantly more observed within the mussel tissue post digestion, which correspond to the results reported by Kolandhasamy et al. (2018). Considering fibres concentrations were 70% of the total available particle shapes, this is not surprising. It is noted, however, that pink and blue spheres were absent from the PF and/or F at low and medium concentrations, suggesting that smaller particles (closer to the preferred size range of phytoplankton ingested by the blue mussel) may be more prominently retained within the shell cavity of the blue mussel. MPs (fibres, pink and blue spheres combined) were observed in the PF and F at all concentrations, indicating that rejection and elimination of particles did occur.

Reflecting on the results of these two studies, researchers should be cautious when stating the blue mussel is likely a good indicator of MP pollution for the marine environment. Desforges et al. (2014) determined the mean number of MPs within the northeastern Pacific Ocean was 2,080 (± 2,190 SD) particles/m^3, reaching almost 10,000 particles/m^3 (Desforges et al., 2014) in coastal waters. A study conducted off the coast of China found similar results. The authors of this study reported average concentrations of 4,137 (± 2462) particles/m^3 along the Yangtze estuary system, reaching values as high as 10,200 particles/m^3 (Zhao et al., 2014). These are the highest values of MPs in seawater recorded globally to date (Gago et al., 2018). This indicates that MP contamination within BC waters is present at similar values established globally, however, the number of particles observed within the mussels were very low (<1 particle / individual after correction and polymer identification), suggesting MP accumulation did not occur over the 60-day period.

It is acknowledged that the 60-day period utilized for the caged mussel study was unable to capture seasonal changes in MP abundances. The authors of the caged study along the coast of Scotland were able to detect significant differences in mean MP abundances per mussel ($p = p < 0.0001$, Fisher LSD). Cages were located in various locations for a one year period and included two winter sampling sessions, suggesting MP abundances either change with the season (Catarino et al., 2018), or differences in mussel filtration rates affect exposure (as mussels filter large volumes in warmer waters (Jorgensen et al., 1990)).

Under optimal conditions, however, the blue mussel is capable of filtering 0.39 L/hr/g of seawater (Foster-Smith, 1975). British Columbia coastal waters have an

average of 2.08 (± 2.19 SD) MPs/L of water. The values of SMPs reported for this equate to less than one particle per individual, indicating that mussels present a snap shot of exposure, rather than the accumulation of particles over a specified time period. Van Cauwenberghe et al. (2015b) hypothesize that an average mussel is roughly 2L/hr and filters roughly 12 hours per day. In total the authors estimate that 24L of seawater are filtered daily, suggesting that mussels are likely exposed to 10 MPs/day (based on estimated concentrations of 0.4 ± 0.3 MPs/L). Again, the notion that MP exposure is constant, should be reflected in MP abundances within the shell cavity of blue mussels if accumulation were occurring.

It appears, that at current contamination levels within the British Columbia marine environment, the blue mussel can reject and eliminate MPs ingested. Like a conveyor belt, particles appear to be brought into and removed from the shell cavity in a steady state. The mechanism for this phenomenon should be investigated further, to determine if, and when, particles are removed from the shell cavity. A chronic exposure experiment, mimicking environmental plastic loads will help researchers further define the mechanisms underlying particle fate post filtration.

Compared to other species, blue mussels appear to have relatively little MP material present within the shell cavity. British Columbia researchers have reported 0.07 – 5.47 MPs/g for clams (*Venerupis philippinarum*) with fibres being the dominant particle type reported (90%; Davidson and Dudas, 2016). Researchers in China examining oysters (*Saccostrea cucullata*) within the Pearl River Estuary determined individuals had 1.5 – 7.2 MPs/g wet weight, with urban oysters containing significantly more particles than those in remote areas. Again, fibres were the dominant particle type reported at 69.4% (Li et al., 2018). Finally, a recent study conducted in the UK examined the potential for trophic transfer of MPs by looking at abundances in captive grey seals (*Halichoerus grypus*) and wild mackerel (*Scomber scombrus*), the main fish species fed to the individuals examined. It was determined that a quarter of the fish contained MPs in their digestive tract, while half of all scat samples contained MPs. The authors conclude that there is the potential for trophic transfer of MPs to top predators that consume the whole body of their prey (Nelms et al., 2018). Again, however, it is important to note that methodology used to determine MP abundances differs greatly between studies, making direct comparison on the number of particles reported within different species difficult to interpret.

It is interesting to consider the high proportion of particles observed in the mussel 24-hours post exposure, compared to the proportion of plastics seen in the PF and F. It is likely that the acute exposure period was too short to fully understand particle fate post filtration, and it is recommended that a similar study be conducted using weathered (Bråte et al., 2018), environmentally relevant concentrations with a chronic exposure mechanism. If similar results persist, it is possible that predicted future concentrations of MPs in seawater will impact mussels and burden their filtration mechanism. The higher number of plastics within the water column may then be too great to reject and/or eliminate the particles consistently. Accumulation may then occur if the burden becomes too large for the mussels to continually expel the particles that are chronically present within the marine environment. A realistic scenario considering plastic pollution is predicted to increase by an order of magnitude by 2025 and mismanaged waste inputs are unlikely to peak before 2100 if management tools are not implemented (Jambeck et al., 2015).

Blue mussels may not be accumulating MPs ranging in size from 25μm – 5mm at current contamination levels within British Columbia, however, individuals are chronically exposed to anthropogenic particles of little nutritional value. Filtering high volumes of particles low in nutrition likely impacts individuals with potential community and population effects. MPs are one of a myriad of anthropogenic stressors posed on mussel populations inclusive of climate change, increasing ocean acidification, and contaminants (Gaylord and Al, 2015; Harley et al., 2006; Smith et al., 2006; Sunday et al., 2011). It is recommended that the nutritional burden imposed by filtering MPs and other anthropogenic particles be studied as the loss of nutrition may impact growth and/or reproductive behaviour.

It is important to realize the lower limit of MPs detected within this study is 25 μm. No significant differences were observed in the number of SMPs between sites or time periods for the field study for MPs ranging in size from 25 μm – 5 mm (with one exception at T60 – Powell River). It is possible, however, that different results would emerge when examining the lowest size limit of MPs available to the blue mussel (1 μm – 24.9 μm). Research suggests that particles within this size fraction increase exponentially in abundance as size decreases (Andrady, 2017). Furthermore, particles within this size range are small enough to translocate to tissues and partition into lipid membranes (Dawson et al., 2018; Rossi et al., 2014; Von Moos et al., 2012). It is

suggested that accumulation occurs within this size range and impose greater health risks (Amaral-Zettler et al., 2016). This is also supported by the by the observation that microfibers were present within the pores of some 20µm filter papers examined in the caged mussel study.

Technological improvements are needed to automate the identification of MPs and quantify particles to the smallest size fractions (Shim et al., 2017). Improvements will increase identification efficiencies and remove user bias from particle identification (Cincinelli et al., 2017; Shim et al., 2017). Technologies exist, however, they are often expensive and pose difficulties when scanning complex matrices (Lusher et al., 2017, 2016; Shim et al., 2016).

Considering the rigorous contamination control exhibited and polymer types identified through FTIR analysis, the application of mussels as indicators of MP pollution for particles ranging in size form 25µm – 5mm in the marine environment is not recommended. The results of this study support the notion that MPs do not accumulate within the shell cavity of these individuals. Instead, a snap shot on the proportion of MPs within the marine environment are provided, detailing exposure rates in real-time. Baseline contamination rates within the species still requires global assessment, however, as this data is important to compare contamination rates over time (assuming standardized techniques are established). Impacts to blue mussel health is an important research area, as it will help define the potential for trophic transfer within the food web (Guzzetti et al., 2018) and potential risk to human health.

References

Amaral-Zettler, L., Andrady, A., Dudas, S., Fabres, J., Galgani, F., Hardesty, D., Hidalgo-Ruz, V., Hong, S., Kershaw, P., Lebreton, L., Lusher, A., Narayan, R., Pahl, S., Potemra, J., Rochman, C., Sherif, S.A., Seager, J., Shim, W.J., Sobral, P., Takada, S., Brink, P. ten, Thiel, M., Thompson, R., Turra, A., Cauwenberghe, L. Van, Sebille, E. van, Vethaak, D., Watkins, E., Wyles, K., Wilcox, C., Zettler, E., Ziveri, P., 2016. Sources, fate and effects of microplastics in the marine environment: Part 2 of a global assessment. Reports Stud. GESAMP 93, 217. doi:10.13140/RG.2.1.3803.7925

Andrady, A.L., 2017. The plastic in microplastics : A review. Mar. Pollut. Bull. 119, 12–22. doi:10.1016/j.marpolbul.2017.01.082

Andrady, A.L., 2011. Microplastics in the marine environment. Mar. Pollut. Bull. 62, 1596–1605. doi:10.1016/j.marpolbul.2011.05.030

Avio, C.G., Gorbi, S., Regoli, F., 2015. Experimental development of a new protocol for extraction and characterization of microplastics in fish tissues: First observations in commercial species from Adriatic Sea. Mar. Environ. Res. 111, 18–26. doi:10.1016/j.marenvres.2015.06.014

Bayne, B., Hawkins, A., Navarro, E., 1987. Feeding and digestion by the mussel *Mytilus edulis* L.(Bivalvia: Mollusca) in mixtures of silt and algal cells at low concentrations. J. Exp. Mar. Bio. Ecol. 111, 1–22.

Bayne, B., Iglesias, I., Hawkins, Aj., Navarrcy, E., HERALt, M., Deslous-paolp, J., 1993. Feeding Behaviour of the Mussel, *Mytilus Edulis*: Responses To Variations in Quantity and Organic Content of the Seston. mar. biol. Ass. U.K 73, 813–829. doi:10.1017/S0025315400034743

Boucher, J. and Friot D. (2017). Primary Microplastics in the Oceans: A Global Evaluation of Sources. Gland, Switzerland: IUCN. 43pp.

Bråte, I.L.N., Blázquez, M., Brooks, S.J., Thomas, K. V., 2018. Weathering impacts the uptake of polyethylene microparticles from toothpaste in Mediterranean mussels (*M. galloprovincialis*). Sci. Total Environ. 626, 1310–1318. doi:10.1016/j.scitotenv.2018.01.141

Brooks, S.J., Harman, C., Hultman, M.T., Berge, J.A., 2015. Integrated biomarker assessment of the effects of tailing discharges from an iron ore mine using blue mussels (*Mytilus* spp.). Sci. Total Environ. 524–525, 104–114. doi:10.1016/j.scitotenv.2015.03.135

Browne, M. a, Galloway, T., Thompson, R., 2007. Microplastic—An Emerging Contaminant of Potential Concern? Integr. Environ. Assess. Manag. — 3, 559–566. doi:10.1002/ieam.5630030215

Browne, M.A., Dissanayake, A., Galloway, T.S., Lowe, D.M., Thompson, R.C., 2008. Ingested Microscopic PlasticTranslocates to the Circulatory System of the Mussel, *Mytilus edulis* (L.). Environ. Sci. Technol 42, 5026–5031. doi: 10.1002/ieam.5630030215

Carr, S.A., 2017. Sources and dispersive modes of micro-fibers in the environment. Integr. Environ. Assess. Manag. 13, 466–469. doi:10.1002/ieam.1916

Catarino, A.I., Macchia, V., Sanderson, W.G., Thompson, R.C., Henry, T.B., 2018. Low levels of microplastics (MP) in wild mussels indicate that MP ingestion by humans is minimal compared to exposure via household fibres fallout during a meal. Environ. Pollut. 237, 675–684. doi:10.1016/j.envpol.2018.02.069

Catarino, A.I., Thompson, R., Sanderson, W., Henry, T.B., 2017. Development and optimization of a standard method for extraction of microplastics in mussels by enzyme digestion of soft tissues. Environ. Toxicol. Chem. 36, 947–951. doi:10.1002/etc.3608

Cincinelli, A., Scopetani, C., Chelazzi, D., Lombardini, E., Martellini, T., Katsoyiannis, A., Fossi, M.C., Corsolini, S., 2017. Microplastic in the surface waters of the Ross Sea (Antarctica): Occurrence, distribution and characterization by FTIR. Chemosphere 175, 391–400. doi:10.1016/j.chemosphere.2017.02.024

Claessens, M., Van Cauwenberghe, L., Vandegehuchte, M.B., Janssen, C.R., 2013. New techniques for the detection of microplastics in sediments and field collected organisms. Mar. Pollut. Bull. 70, 227–233. doi:10.1016/j.marpolbul.2013.03.009

Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C., Galloway, T.S., 2016. Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. Environ. Sci. Technol. 50, 3239–3246. doi:10.1021/acs.est.5b05905

Cole, M., Webb, H., Lindeque, P.K., Fileman, E.S., Halsband, C., Galloway, T.S., 2014. Isolation of microplastics in biota-rich seawater samples and marine organisms. Sci. Rep. 4, 4528. doi:10.1038/srep04528

Comnea-stancu, I.R., Wieland, K., Ramer, G., Schwaighofer, A., Lendl, B., 2017. On the Identification of Rayon / Viscose as a Major Fraction of Microplastics in the Marine Environment : Discrimination between Natural and Manmade Cellulosic Fibers Using Fourier Transform Infrared Spectroscopy 71, 939–950. doi:10.1177/0003702816660725

Courtene-Jones, W., Quinn, B., Murphy, F., Gary, S.F., Narayanaswamy, B.E., 2016. Optimisation of enzymatic digestion and validation of specimen preservation methods for the analysis of ingested microplastics. Anal. Methods 9, 1437–1445. doi:10.1039/C6AY02343F

Davidson, K., Dudas, S.E., 2016. Microplastic Ingestion by Wild and Cultured Manila Clams (V*enerupis philippinarum*) from Baynes Sound, British Columbia. Arch. Environ. Contam. Toxicol. doi:10.1007/s00244-016-0286-4

Dawson, A.L., Kawaguchi, S., King, C.K., Townsend, K.A., King, R., Huston, W.M., Bengtson Nash, S.M., 2018. Turning microplastics into nanoplastics through digestive fragmentation by Antarctic krill. Nat. Commun. 9, 1001. doi:10.1038/s41467-018-03465-9

De Witte, B., Devriese, L., Bekaert, K., Hoffman, S., Vandermeersch, G., Cooreman, K., Robbens, J., 2014. Quality assessment of the blue mussel (*Mytilus edulis*): Comparison between commercial and wild types. Mar. Pollut. Bull. 85, 146–155. doi:10.1016/j.marpolbul.2014.06.006

Desforges, J.P., Galbraith, M., Ross, P.S., 2015. Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. Arch Env. Contam Toxicol 69, 320–330. doi:10.1007/s00244-015-0172-5\r10.1007/s00244-015-0172-5 [pii]

Desforges, J.P.W., Galbraith, M., Dangerfield, N., Ross, P.S., 2014. Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. Mar. Pollut. Bull. 79, 94–99. doi:10.1016/j.marpolbul.2013.12.035

Dris, R., Gasperi, J., Saad, M., Mirande, C., Tassin, B., 2015. Synthetic fibers in atmospheric fallout: A source of microplastics in the environment? Mar. Pollut. Bull. 4–7. doi:10.1016/j.marpolbul.2016.01.006

Duis, K., Coors, A., 2016. Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environ. Sci. Eur. 28, 1–25. doi:10.1186/s12302-015-0069-y

Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3. doi:10.1016/j.envpol.2013.01.046

Farrington, J.W., Tripp, B.W., Tanabe, S., Subramanian, A., Sericano, J.L., Wade, T.L., Knap, A.H., 2016. Edward D. Goldberg's proposal of "the Mussel Watch": Reflections after 40years. Mar. Pollut. Bull. 110, 501–510. doi:10.1016/j.marpolbul.2016.05.074

Foekema, E.M., De Gruijter, C., Mergia, M.T., van Franeker, J.A., Murk, A.J., Koelmans, A. a, 2013. Plastic in North Sea Fish. Environ. Sci. Technol. 47. doi:10.1021/es400931b

Foster-Smith, R.L., 1975. The Effect of Concentration of Suspension on the Filtration Rates of Pseudofaecal Production for *Mytilus eduli*s L. *Cerastoderma edule* (L.) and *Venerupis pullastra* (Montagu). J. Exp. Mar. Bio. Ecol. 17, 1–22.

Gadzała-Kopciuch, R., Berecka, B., Bartoszewicz, J., Buszewski, B., 2004. Some considerations about bioindicators in environmental monitoring. Polish J. Environ. Stud. 13, 453–462. doi:10.1.1.470.4808

Gago, J., Carretero, O., Filgueiras, A. V., Viñas, L., 2018. Synthetic microfibers in the marine environment: A review on their occurrence in seawater and sediments. Mar. Pollut. Bull. 127, 365–376. doi:10.1016/j.marpolbul.2017.11.070

Gaylord, B., Al, E.T., 2015. Ocean acidification through the lens of ecological theory. Ecology. 96 (1), 3–15.

GESAMP (2015). “Sources, fate and effects of microplastics in the marine environment: a global assessment” (Kershaw, P. J., ed.). (IMO/FAO/UNESCO-IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection). Rep. Stud. GESAMP No. 90, 96 p.

Geyer, R., Jambeck, J.R., Law, K.L., 2017. Production, use, and fate of all plastics ever made. Sci. Adv. 3, 1-5. doi:10.1126/sciadv.1700782

Gies, E.A., LeNoble, J.L., Noël, M., Etemadifar, A., Bishay, F., Hall, E.R., Ross, P.S., 2018. Retention of microplastics in a major secondary wastewater treatment plant in Vancouver, Canada. Mar. Pollut. Bull. 133, 553–561. doi:10.1016/j.marpolbul.2018.06.006

Gigault, J., Halle, A. ter, Baudrimont, M., Pascal, P.Y., Gauffre, F., Phi, T.L., El Hadri, H., Grassl, B., Reynaud, S., 2018. Current opinion: What is a nanoplastic? Environ. Pollut. doi:10.1016/j.envpol.2018.01.024

Giltrap, M., Ronan, J., Hardenberg, S., Parkes, G., McHugh, B., McGovern, E., Wilson, J.G., 2013. Assessment of biomarkers in *Mytilus eduli*s to determine good environmental status for implementation of MSFD in Ireland. Mar. Pollut. Bull. 71, 240–249. doi:10.1016/j.marpolbul.2013.02.021

Goldberg, E.D., 1975. The mussel watch - A first step in global marine monitoring. Mar. Pollut. Bull. 6, 111. doi:10.1016/0025-326X(75)90271-4

Google Earth. 2018. 7.1.8.3036 (32-bit). Map of caged mussel study area centered around Vancouver, British Columbia. 49°15’14.04”N, 122°57’19.58”W. elevation 112m. Accessed 28 August 2018

Green, D.S., Boots, B., Thompson, R., 2017. Microplastics Affect the Ecological Functioning of an Important Biogenic Habitat. Environ. Sci. Technol. 51. doi:10.1021/acs.est.6b04496

Guzzetti, E., Sureda, A., Tejada, S., Faggio, C., 2018. Microplastic in Marine Organism: Environmental and Toxicological Effects. Environ. Toxicol. Pharmacol. 64, 164–171. doi:10.1016/j.etap.2018.10.009

Hale, R.C., 2017. Analytical challenges associated with the determination of microplastics in the environment. Anal. Methods 00, 1–2. doi:10.1039/c7ay90015e

Harley, C.D.G., 2011. Climate Change, Keystone Predation, and Biodiversity Loss. Science. 334, 1124–1127.

Harley, C.D.G., Randall Hughes, A., Hultgren, K.M., Miner, B.G., Sorte, C.J.B., Thornber, C.S., Rodriguez, L.F., Tomanek, L., Williams, S.L., 2006. The impacts of climate change in coastal marine systems: Climate change in coastal marine systems. Ecol. Lett. 9, 228–241. doi:10.1111/j.1461-0248.2005.00871.x

Heath, D.D., Rawson, P.D., Hilbish, T.J., 1995. PCR-based nuclear markers identify alien blue mussel *Mytilus* spp. genotypes on the west coast of Canada. Can. J. Fish. Aquat. Sci. 52, 2621–2627. doi:10.1111/j.1740-8261.2008.00384.x

Helmuth, B. et al. 2016. Data Descriptor : Long-term, high frequency in situ measurements of intertidal mussel bed temperatures using biomimetic sensors. Sci. Data 3:160087 doi: 10.1038/sdata.2016.87.

Hidalgo-Ruz, V., Gutow, L., Thompson, R.C., Thiel, M., 2012. Microplastics in the marine environment: A review of the methods used for identification and quantification. Environ. Sci. Technol. 46, 3060–3075. doi:10.1021/es2031505

Holt, E.A., Miller, S.W., 2011. How do we assess the impacts of human activities on natural ecosystems ? What can the biota tell us about the environment and its response to natural stress ? Introduction What Is a Bioindicator ? Nat. Educ. Knowl. 1–10.

Jahnke, A., Arp, H.P.H., Escher, B.I., Gewert, B., Gorokhova, E., Ku, D., Ogonowski, M., Pottho, A., Rummel, C., Schmitt-jansen, M., Toorman, E., Macleod, M., 2017. Impacts of Weathering Plastic in the Marine Environment. Environ. Sci. Technol. Lett. 4, 85–90. doi:10.1021/acs.estlett.7b00008

Jambeck, J.R., Geyer, R., Wilcox, C., Siegler, T.R., Perryman, M., Andrady, A., Narayan, R., Law, K.L., 2015. Plastic waste inputs from land into the ocean. Science. 347, 768–771.

Jorgensen, B.C., Larsen, P.S., Riisgard, H.U., 1990. Effects of temperature on the mussel pump 64, 89–97.

Kolandhasamy, P., Su, L., Li, J., Qu, X., Jabeen, K., Shi, H., 2018. Adherence of microplastics to soft tissue of mussels: A novel way to uptake microplastics beyond ingestion. Sci. Total Environ. 610–611, 635–640. doi:10.1016/j.scitotenv.2017.08.053

Lambert, S., Scherer, C., Wagner, M., 2017. Ecotoxicity testing of microplastics: Considering the heterogeneity of physicochemical properties. Integr. Environ. Assess. Manag. 13, 470–475. doi:10.1002/ieam.1901

Li, H.X., Ma, L.S., Lin, L., Ni, Z.X., Xu, X.R., Shi, H.H., Yan, Y., Zheng, G.M., Rittschof, D., 2018. Microplastics in oysters *Saccostrea cucullata* along the Pearl River Estuary, China. Environ. Pollut. 236, 619–625. doi:10.1016/j.envpol.2018.01.083

Li, J., Qu, X., Su, L., Zhang, W., Yang, D., Kolandhasamy, P., Li, D., Shi, H., 2016. Microplastics in mussels along the coastal waters of China. Environ. Pollut. 214, 177–184. doi:10.1016/j.envpol.2016.04.012

Li, J., Yang, D., Li, L., Jabeen, K., Shi, H., 2015. Microplastics in commercial bivalves from China. Environ. Pollut. 207, 190–195. doi:10.1016/j.envpol.2015.09.018

Luoma, S.N., 1996. The developing framework of marine ecotoxicology: Pollutants as a variable in marine ecosystems? J. Exp. Mar. Bio. Ecol. 200, 29–55. doi:10.1016/S0022-0981(96)02679-2

Lusher, A., Bråte, I.L.N., Hurley, R., Iversen, K., Olsen, M., 2017. Testing of methodology for measuring microplastics in blue mussels (*Mytilus* spp) and sediments, and recommendations for future monitoring of microplastics (R & D-project). NIVA-report 7209-2017 7, 87.

Lusher, A.L., McHugh, M., Thompson, R.C., 2013. Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Mar. Pollut. Bull. 67, 94–99. doi:10.1016/j.marpolbul.2012.11.028

Lusher, A.L., Tirelli, V., O'Connor, I., Officer, R., 2015. Microplastics in Arctic polar waters: The first reported values of particles in surface and sub-surface samples. Sci. Rep. 5, 1–9. doi:10.1038/srep14947

Lusher, A.L., Welden, N.A., Sobral, P., Cole, M., 2016. Sampling , isolating and identifying microplastics ingested by fish and invertebrates : a critical review. Anal. Methods 00, 1–15. doi:10.1039/C6AY02415G

Mai, L., Bao, L.-J., Shi, L., Wong, C.S., Zeng, E.Y., 2018. A review of methods for measuring microplastics in aquatic environments. Environ. Sci. Pollut. Res. doi:10.1007/s11356-018-1692-0

Marine & Environmental Research Institute, 2015. Guide to Microplastic Identification.

Mathalon, A., Hill, P., 2014. Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. Mar. Pollut. Bull. 81, 69–79. doi:10.1016/j.marpolbul.2014.02.018

McPhee, D., Duhaime, J., Tuen, A., Parsons, G.J., 2017. Canadian Aquaculture R&D Review. Aquaculture Association of Canada Special Publication 25.

Mitacs. 2016. Mitacs Accelerate Application. Microplastic pollution in coastal British Columbia: a baseline assessment of contamination in blue mussels. Ocean Wise Conservation Association.

Nelms, S.E., Galloway, T.S., Godley, B.J., Jarvis, D.S., Lindeque, P.K., 2018. Investigating microplastic trophic transfer in marine top predators. Environ. Pollut. 238, 999–1007. doi:10.1016/j.envpol.2018.02.016

Pales Espinosa, E., Cerrato, R.M., Wikfors, G.H., Allam, B., 2016. Modeling food choice in the two suspension-feeding bivalves, *Crassostrea virginica* and *Mytilus edulis.* Mar. Biol. 163, 1–13. doi:10.1007/s00227-016-2815-0

Peeken, I., Primpke, S., Beyer, B., Gütermann, J., Katlein, C., Krumpen, T., Bergmann, M., Hehemann, L., Gerdts, G., 2018. Arctic sea ice is an important temporal sink and means of transport for microplastic. Nat. Commun. 9. doi:10.1038/s41467-018-03825-5

Pfister, C.A., Roy, K., Wootton, J.T., Mccoy, S.J., Paine, R.T., Suchanek, T.H., Sanford, E., 2016. Historical baselines and the future of shell calcification for a foundation species in a changing ocean. Proc. R. Soc. B Biol. Sci. 283, 20160392. doi:10.1098/rspb.2016.0392

Phuong, N.N., Zalouk-Vergnoux, A., Kamari, A., Mouneyrac, C., Amiard, F., Poirier, L., Lagarde, F., 2017. Quantification and characterization of microplastics in blue mussels (*Mytilus edulis*): protocol setup and preliminary data on the contamination of the French Atlantic coast. Environ. Sci. Pollut. Res. doi:10.1007/s11356-017-8862-3

PlasticsEurope, 2017. Plastics – the Facts 2017. doi:10.1016/j.marpolbul.2013.01.015

Potthoff, A., Oelschlägel, K., Schmitt-Jansen, M., Rummel, C.D., Kühnel, D., 2017. From the sea to the laboratory: Characterization of microplastic as prerequisite for the assessment of ecotoxicological impact. Integr. Environ. Assess. Manag. 13, 500–504. doi:10.1002/ieam.1902

Qu, X., Su, L., Li, H., Liang, M., Shi, H., 2018. Assessing the relationship between the abundance and properties of microplastics in water and in mussels. Sci. Total Environ. 621, 679–686. doi:10.1016/j.scitotenv.2017.11.284

Regional District Nanaimo (RDN). 2018. Information About The Bowser Village Centre Wastewater Project.

Renzi, M., Guerranti, C., Blašković, A., 2018. Microplastic contents from maricultured and natural mussels. Mar. Pollut. Bull. 131, 248–251. doi:10.1016/j.marpolbul.2018.04.035

Rocha-Santos, T., Duarte, A.C., 2015. A critical overview of the analytical approaches to the occurrence, the fate and the behavior of microplastics in the environment. TrAC - Trends Anal. Chem. 65, 47–53. doi:10.1016/j.trac.2014.10.011

Rochman, C.M., Browne, M.A., Halpern, B.S., Hentschel, B.T., Hoh, E., Karapanagioti, H.K., Rios-Mendoza, L.M., Takada, H., Teh, S., Thompson, R.C., 2013. Policy: Classify plastic waste as hazardous. Nature 494, 169–71. doi:10.1038/494169a

Rochman, C.M., Regan, F., Thompson, R.C., 2017. On the harmonization of methods for measuring the occurrence, fate and effects of microplastics. Anal. Methods 9, 1324–1325. doi:10.1039/C7AY90014G

Rochman, C.M., Tahir, A., Williams, S.L., Baxa, D. V, Lam, R., Miller, J.T., Teh, F.-C., Werorilangi, S., Teh, S.J., 2015. Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. Sci. Rep. 5, 14340. doi:10.1038/srep14340

Rossi, G., Barnoud, J., Monticelli, L., 2014. Polystyrene nanoparticles perturb lipid membranes. J. Phys. Chem. Lett. 5, 241–246. doi:10.1021/jz402234c

Schmidt, D., 1999. A Review of California Mussel (*Mytilus californianus*) Fisheries Biology and Fisheries Programs.

Shim, W.J., Hong, H., Eo, S., 2017. Identification methods in microplastic analysis : Anal. Methods 9, 1384–1391. doi:10.1039/C6AY02558G

Smith, J.R., Fong, P., Ambrose, R.F., 2006. Dramatic Declines in Mussel Bed Community Diversity: Response to Climate Change? Ecology. 87, 1153–1161.

Smith, M., Love, D.C., Rochman, C.M., Neff, R.A., 2018. Microplastics in Seafood and the Implications for Human Health. Curr. Environ. Heal. Reports 1–12. doi:10.1007/s40572-018-0206-z

Sunday, J.M., Crim, R.N., Harley, C.D.G., Hart, M.W., 2011. Quantifying rates of evolutionary adaptation in response to ocean acidification. PLoS One 6, 1–8. doi:10.1371/journal.pone.0022881

Thompson, R.C., Rowland, S.J., Russell, A.E., Olsen, Y., Mitchell, R.P., Davis, A., Rowland, S.J., John, A.W.G., Mcgonigle, D., Russell, A.E., 2004. Lost at Sea: Where Is All the Plastic? Science. 304, 834 doi:10.1126/science.1094559

Van Cauwenberghe, L., Claessens, M., Vandegehuchte, M.B., Janssen, C.R., 2015a. Microplastics are taken up by mussels (*Mytilus edulis*) and lugworms (*Arenicola marina*) living in natural habitats. Environ. Pollut. 199, 10–17. doi:10.1016/j.envpol.2015.01.008

Van Cauwenberghe, L., Devriese, L., Galgani, F., Robbens, J., Janssen, C.R., 2015b. Microplastics in sediments: A review of techniques, occurrence and effects. Mar. Environ. Res. 111, 5–17. doi:10.1016/j.marenvres.2015.06.007

Van Cauwenberghe, L., Janssen, C.R., 2014. Microplastics in bivalves cultured for human consumption. Environ. Pollut. 193, 65–70. doi:10.1016/j.envpol.2014.06.010

van der Schatte Olivier, A., Jones, L., Vay, L. Le, Christie, M., Wilson, J., Malham, S.K., 2018. A global review of the ecosystem services provided by bivalve aquaculture. Rev. Aquac. 1–23. doi:10.1111/raq.12301

Von Moos, N., Burkhardt-Holm, P., Köhler, A., 2012. Uptake and effects of microplastics on cells and tissue of the blue mussel *Mytilus edulis* L. after an experimental exposure. Environ. Sci. Technol. 46, 11327–11335. doi:10.1021/es302332w

Wang, W., Ndungu, A.W., Li, Z., Wang, J., 2017. Microplastics pollution in inland freshwaters of China: A case study in urban surface waters of Wuhan, China. Sci. Total Environ. 575, 1369–1374. doi:10.1016/j.scitotenv.2016.09.213

Widdows, J., Fieth, P., Worrall, C.M., 1979. Relationships between seston, available food and feeding activity in the common mussel *Mytilus edulis*. Mar. Biol. 50, 195–207. doi:10.1007/BF00394201

Woodall, L.C., Gwinnett, C., Packer, M., Thompson, R.C., Robinson, L.F., Paterson, G.L.J., 2015. Using a forensic science approach to minimize environmental contamination and to identify microfibres in marine sediments. Mar. Pollut. Bull. 95, 40–46. doi:10.1016/j.marpolbul.2015.04.044

Woodall, L.C., Sanchez-Vidal, A., Canals, M., Paterson, G.L.J., Coppock, R., Sleight, V., Calafat, A., Rogers, A.D., Narayanaswamy, B.E., Thompson, R.C., 2014. The deep sea is a major sink for microplastic debris. R. Soc. Open Sci. 1, 140317–140317. doi:10.1098/rsos.140317

Wright, S.L., Thompson, R.C., Galloway, T.S., 2013. The physical impacts of microplastics on marine organisms: A review. Environ. Pollut. 178, 483–492. doi:10.1016/j.envpol.2013.02.031

Zalasiewicz, J., Waters, C.N., Ivar do Sul, J.A., Corcoran, P.L., Barnosky, A.D., Cearreta, A., Edgeworth, M., Gałuszka, A., Jeandel, C., Leinfelder, R., McNeill, J.R., Steffen, W., Summerhayes, C., Wagreich, M., Williams, M., Wolfe, A.P., Yonan, Y., 2016. The geological cycle of plastics and their use as a stratigraphic indicator of the Anthropocene. Anthropocene 13, 4–17. doi:10.1016/j.ancene.2016.01.002

Zhao, S., Zhu, L., Wang, T., Li, D., 2014. Suspended microplastics in the surface water of the Yangtze Estuary System, China: First observations on occurrence, distribution. Mar. Pollut. Bull. 86, 562–568. doi:10.1016/j.marpolbul.2014.06.032

Appendix A.

Mussel condition and suspected microplastics observed in Caged Mussel Study

Table A1 **Mean body condition index (BCI) and mean length (cm) for mussel batches (n = 5 – 8 mussels per site/time period) from 12 locations across 3 sampling periods (T0 = Day 0, T30 = Day 30, T60 = Day 60). BCI [(body wet weight (BWW)/total wet weight (TWW))*100] describes relative nutritional status and general health of the mussels at each sampling period.**

Site	Time Period	BCI [(BWW/TWW) *100]					Length (cm)					
		N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	Range
Powell River	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	37.9	3.3	34.2	43.2	8	37.8	3.1	33.8	42.4	8.6
	T60	8	43.1	10.2	30.6	63.9	8	38.0	3.2	33.7	42.8	9.1
Campbell River	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	7	44.8	12.0	30.5	64.4	7	46.0	2.1	43.1	48.6	5.5
	T60	7	45.5	2.8	41.1	49.8	8	41.2	2.2	38.2	45.7	7.5
Deep Bay	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	42.7	4.8	36.6	50.6	7	43.9	2.2	41.1	47.0	5.9
	T60	5	50.7	4.2	46.0	55.5	5	46.5	2.5	43.7	49.3	5.6
Victoria	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	48.7	4.1	42.5	56.0	8	44.9	3.1	41.1	50.0	8.9
	T60	8	45.6	3.6	40.8	49.4	8	43.6	1.3	42.0	46.3	4.3
Vancouver Aquarium	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	5	39.1	3.1	34.6	42.0	5	42.2	2.5	38.7	44.7	6.0
	T60	8	42.0	4.0	35.9	48.2	8	43.5	2.2	39.9	47.4	7.5
Canada Place	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	7	43.3	2.4	39.8	46.7	7	44.2	3.3	40.7	50.6	9.9
	T60	6	45.6	3.7	40.2	49.1	7	40.3	3.0	37.3	44.9	7.6
Coal Harbour	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	35.9	4.6	30.1	44.0	8	41.7	2.9	38.5	48.1	9.7
	T60	8	45.3	4.3	38.2	51.3	8	44.2	2.3	41.4	48.5	7.1

Site	Time Period	BCI [(BWW/TWW) *100]					Length (cm)					
		N	Mean	SD	Min	Max	N	Mean	SD	Min	Max	Range
Port Moody	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	39.1	6.7	25.4	49.2	8	39.2	1.5	37.7	41.5	3.8
	T60	8	48.2	5.9	40.3	59.4	8	45.6	2.8	41.1	49.1	8.0
Indian Arm - Mid	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	41.9	4.2	37.1	47.5	8	42.1	1.5	40.6	44.5	3.9
	T60	6	48.5	3.9	41.4	51.5	6	45.6	2.3	41.9	48.5	6.6
Indian Arm - North	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	8	43.1	3.6	38.6	48.7	8	44.1	3.0	39.0	48.6	9.6
	T60	7	51.9	4.9	44.9	60.4	8	39.5	1.2	38.0	41.0	3.0
Howe Sound	T0	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T30	7	43.8	2.3	40.4	47.1	8	39.5	1.3	37.1	40.9	3.9
	T60	5	48.6	2.1	46.4	51.3	5	43.6	0.9	42.9	45.1	2.2

.

Table A2 **Summary of total and mean suspected microplastics (SMPs) observed per site/time period (green columns). Individual data was corrected by removing 1.26 SMP/mussel to account for procedural blank contamination. Resulting corrected SMP (CSMP) data provided in yellow columns. All data anaylsis completed using the #CSMP/gram of wet weight tissue (blue columns) to ensure data was standardized for mussel size across all sites and time periods.**

Site	Time Period	Mussels (n)	Original SMP			Corrected SMP			CSMP / GWW Tissue		
			Total	Mean	SD	Total	Mean	SD	Total	Mean	SD
Powell River	T0	8	7	0.88	0.99	1.74	0.22	0.62	0.74	0.09	0.26
	T30	8	11	1.38	1.19	3.48	0.44	0.97	2.57	0.32	0.71
	T60	7	30	4.29	2.93	21.44	3.06	2.88	14.37	2.05	2.01
Campbell River	T30	7	12	1.71	1.25	4.7	0.67	0.59	1.46	0.21	0.24
	T60	8	10	1.25	1.16	3.96	0.50	0.62	1.25	0.18	0.25
Deep Bay	T30	8	22	2.75	2.19	14.44	1.81	1.77	5.46	0.68	0.73
	T60	5	5	1.00	0.71	0.74	0.15	0.33	0.22	0.04	0.10
Victoria	T30	8	19	2.38	1.85	14.44	1.81	1.69	4.91	0.61	0.55
	T60	8	16	2.00	1.69	8.7	1.09	1.29	3.21	0.40	0.48
Vancouver Aquarium	T30	5	6	1.20	1.30	2.48	0.50	0.77	0.99	0.20	0.30
	T60	8	13	1.63	0.52	3.7	0.46	0.38	1.73	0.22	0.19
Coal Harbour	T30	8	10	1.25	0.71	2.22	0.28	0.38	1.25	0.16	0.22
	T60	8	28	3.50	3.55	19.7	2.46	3.34	7.45	0.93	1.51
Canada Place	T30	8	15	1.88	1.36	6.96	0.87	1.07	2.27	0.28	0.34
	T60	8	24	3.00	1.85	14.44	1.81	1.77	5.49	0.78	0.79
Port Moody	T30	8	13	1.63	1.19	5.7	0.71	0.72	3.18	0.40	0.43
	T60	8	25	3.13	2.03	15.44	1.93	1.96	5.26	0.66	0.70
Indian Arm - Mid	T30	8	21	2.63	1.51	12.44	1.56	1.18	5.48	0.68	0.55
	T60	6	10	1.67	1.21	4.22	0.70	0.85	1.16	0.19	0.23
Indian Arm - North	T30	8	13	1.63	1.06	4.96	0.62	0.76	1.60	0.20	0.24
	T60	8	8	1.00	0.93	1.74	0.22	0.62	0.00	0.00	0.00
Howe Sound	T30	8	13	1.63	1.30	5.22	0.65	1.05	1.33	0.17	0.25
	T60	5	5	1.00	1.41	2.48	0.50	0.77	0.96	0.19	0.31
All Sites / Time Periods		**171**	**336**	**1.96**	**1.77**	1.77	**1.03**	**1.52**	**72.35**	**0.43**	**0.76**

Appendix B.

Boxplot of microplastic fate post filtration in mussel exposure experiment

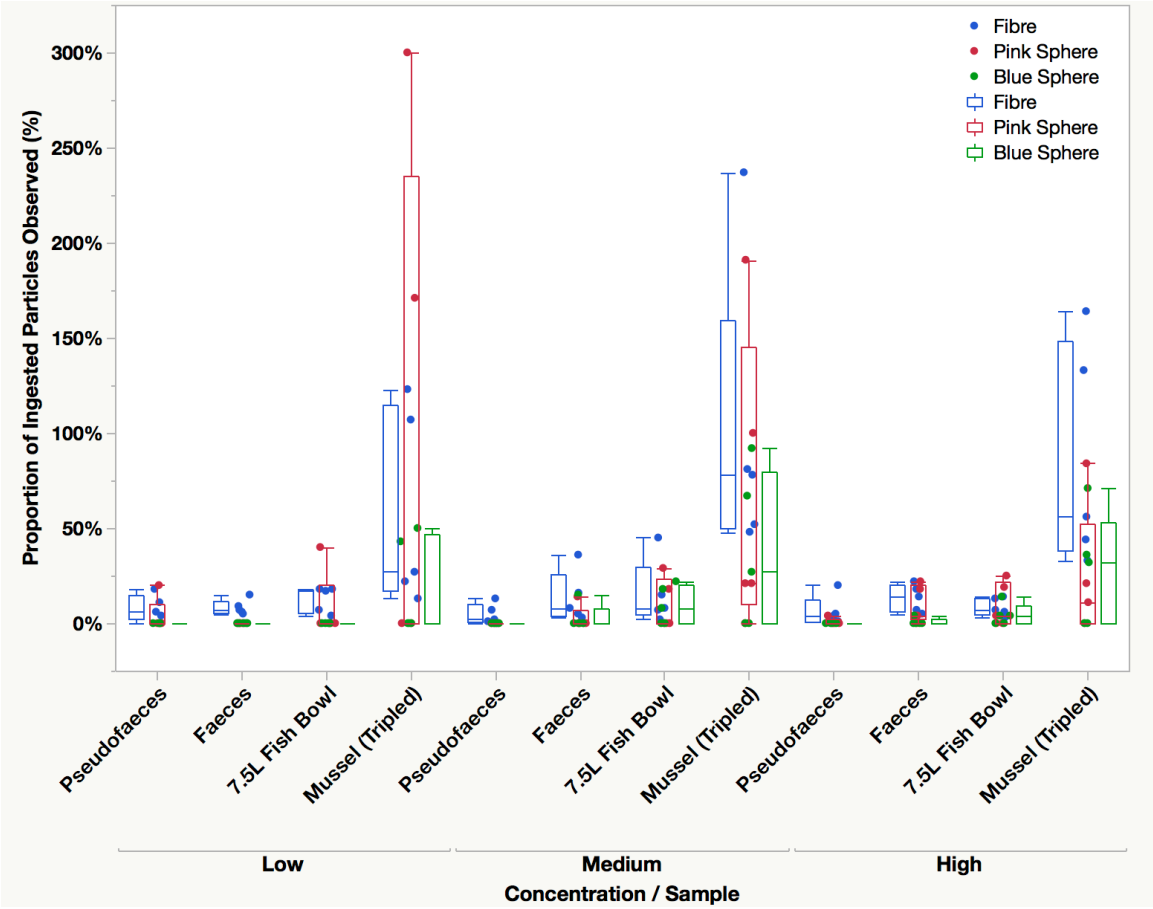

Figure B1 **Boxplot with scatterplot overlaid of the mean proportion of microplastics filtered by the mussels (MPs) for fibres, pink and blue spheres and subsequently observed in the pseudofaeces (PF), faeces (F), 7.5L fish bowl and mussel. Values are provided for the low, medium and high concentration groups. Only one of three mussels was examined for MPs as two were sent for health analysis. Thus, MP values determined in the single mussel were tripled to account for unknown values. For this reason, mussel proportions exceed 100%. Significantly more MPs were observed in the mussel than in the PF, F, or 7.5L fish bowl (p = <0.001). Note that any MPs observed within the 7.5 L fish bowl samples were depurated from the mussel overnight and could not be determined as PF or F and were treated independently.**