The anthropogenic influence of shellfish aquaculture and microplastics on juvenile Pacific salmon on the east coast of Vancouver Island

by

Brenna Collicutt
Bachelor of Science, Vancouver Island University, 2011

A Thesis Submitted in Partial Fulfillment
of the Requirements for the Degree of

MASTER OF SCIENCE

in the Department of Biology

Supervisory Committee

The anthropogenic influence of shellfish aquaculture and microplastics on juvenile Pacific salmon on the east coast of Vancouver Island

by

Brenna Collicutt
Bachelor of Science, Vancouver Island University, 2011

Supervisory Committee

Dr. Francis Juanes, Department of Biology
Supervisor

Dr. Sarah Dudas, Department of Biology
Co-Supervisor

Dr. John Dower, Department of Biology
Departmental Member

Abstract

In the northeast Pacific, salmon are an integral part of ecology, economics and culture. Nearshore areas, where juvenile salmon reside upon leaving their natal streams, are important habitat during a critical time where growth can determine overall survivorship. With the rise in human development in coastal areas, these valuable habitats are becoming increasingly modified, however, the ecological ramifications are not fully understood. This study focuses on two types of anthropogenic influence including shellfish aquaculture, which modifies intertidal areas by adding structures such as intertidal fencing and anti-predator nets, and plastic marine pollution in the form of microplastics. We beach seined at sites within an area extensively modified for shellfish aquaculture (Baynes Sound) to examine juvenile salmon abundance, condition, feeding intensity and prey at aquaculture and non-aquaculture areas. In addition, we also beach seined, and along the east coast of Vancouver Island to determine the incidence of microplastics in juvenile Chinook salmon and their nearshore environments. No significant differences were found between areas in the abundance, diets, condition or feeding intensity of juvenile Coho and Chinook. Chum had different prey and a higher condition and feeding intensity at aquaculture sites, suggesting that species such as Chum feeding on more benthic prey items have a higher probability of being impacted by shellfish aquaculture modifications and in this case we observed positive effects. Microplastic analysis showed juvenile Chinook salmon contained 1.15 ± 1.41 (SD) microplastics per individual while water and sediment samples had 659.88 ± 520.87 microplastics m^{-3} and 60.2 ± 63.4 microplastics kg^{-1} dry weight, respectively. We found no differences in microplastic concentrations in juvenile Chinook and water samples among sites but observed significantly higher concentrations in sediment at our Deep Bay site compared to Nanaimo and Cowichan Bay. These differences may be due to site bathymetry and oceanographic differences facilitating settlement at the Deep Bay site and/or may be a result of differential plastic sources in the area including shellfish farming and a marina. Shellfish aquaculture had negligible or positive effects on juvenile salmon abundance, diet, condition and feeding intensity and Chinook microplastic concentrations were relatively low compared to literature values. Although fitness consequences and ecosystem-wide implications must be addressed in the future, it appears shellfish aquaculture and microplastics are not immediate threats to juvenile Pacific salmon along the east coast of Vancouver Island at this time. However, continued monitoring programs and larger-scale studies should be implemented as shoreline modification and plastic use continues to increase.

Table of Contents

List of Tables

List of Figures

Acknowledgments

I'd like to extend my deepest gratitude to my supervisors Drs. Sarah Dudas and Francis Juanes for taking me on as a student and opening my eyes to a whole new world of ecology and research. I'd also like to thank Vancouver Island University and the University of Victoria that have provided endless resources for this project and to recognize my committee member Dr. John Dower for his insight and assistance throughout this process.

Thanks to my partner in crime along this master's journey, Robert Bourdon and my amazing field and lab crews including Katie Davidson, Aaron Dodd, Matt Miller, Stephen Kinsey, Nathan Hambrook and a number of volunteers. I appreciate all of your assistance, insight, support, laughter and entertainment more than you know. I'd also like to thank the Dudas/Juanes/Baum labs for providing an amazing work environment and giving me the opportunity to work alongside and be inspired by so many brilliant minds.

I'd like to extend my thanks to Dave Switzer and the International Centre for Sturgeon Studies at VIU for allowing me to use their lab facilities and equipment as well as the Department of Fisheries and Oceans and the Pacific Salmon Foundation for their assistance with salmon and plastic identification. Thanks also to Peter Ross, Esther Gies and Ellika Crichton for microplastics guidance and equipment, and for sharing in the frustration that is microplastics method testing.

Thanks to Anne Shaffer and the Coastal Watershed Institute for the use of their beach seine as well as passing on expert knowledge and experience. I'd like to recognize the support of Keith Reid, Brian Yip and Pham Tran for allowing us to work on their shellfish leases, and Brian Kingzett and the Deep Bay Marine Station for providing us with many resources for our work. I'd like to thank funding sources of the Aquaculture Association of Canada, the American Fisheries Society, Environment Canada, VIU, and UVic, without which, this project would not be possible.

Finally, I'd like to thank my rugby family for providing me with an outlet to keep me sane throughout this process and a huge thank you to my family and friends for their continued love and support.

Chapter 1: Introduction

Humans depend on the ocean for a variety of ecosystem services and, as a result, the vast majority of people live near coastal environments (Lotze *et al.* 2006). In doing so, we have exploited marine environments in a variety of ways and continue to threaten their sustainability through means such as overfishing, invasive species introductions, coastal development, pollution and global climate change (Derraik 2002; Kennish 2002; Lotze *et al.* 2006; Halpern *et al.* 2008). Modifications to coastal ecosystems for residential, recreational and commercial purposes may be impacting the marine species they sustain (Bulleri and Chapman 2010). A growing body of evidence supports the importance of estuarine and coastal ecosystems (Lotze *et al.* 2006; Halpern *et al.* 2008; Bulleri and Chapman 2010) including an emphasis on these areas as critical fish habitat and nurseries (Beck *et al.* 2003; Able 2005) for many ecologically important species such as Pacific salmon (Simenstad *et al.* 1982).

1.1. Pacific salmon and nearshore areas

Pacific salmon (*Oncorhynchus* sp.) hold immense ecological, economic and cultural importance in the northeast Pacific and are one of the most valuable commercial and recreational fishery resources there (Quinn 2005). Traditionally, Pacific salmon are an important part of coastal First Nation's culture, nutrition, and commerce and hold a strong iconic significance for most inhabitants and visitors to the area. Pacific salmon are considered a keystone species in many northeast Pacific ecosystems in addition to being integral parts of several food webs. Through their anadromous and semelparous lifecycle, Pacific salmon also play key roles in both aquatic and terrestrial environments and provide a necessary link for the distribution of nutrients throughout coastal ecosystems (Willson and Hulupka 1995).

Pacific salmon declines since the 1970s (particularly Chinook and Coho) have been attributed to over-fishing, habitat loss and low marine survival (DFO 1991, 1999; Labelle *et al.* 1997; Beamish *et al.* 2008). Because of the link between total survival in the marine environment and survival during the first marine year, an emphasis has been placed on factors related to mortality during juvenile salmon early marine residence in coastal areas (Beamish and Mahnken 2001; Beamish *et al.* 2010).

Estuarine and nearshore areas provide an integral link between freshwater and marine systems for juvenile salmon. A growing body of literature supports the vital importance of maintaining these areas as they are essential for the longevity of Pacific salmon populations (Healey 1982; Simenstad et al. 1982; Magnusson and Hilborn 2003; Bottom et al. 2005; Fresh 2006). Although the fundamental requirements of estuaries to support Pacific salmon are similar, each species and different populations will vary in their dependence and use of these areas (Simenstad *et al.* 1982; Fresh 2006). For example, Chinook are generally considered to be the most dependent on and will use estuaries for the longest duration (relative to other salmon species) with residence times ranging from 6 to 29 weeks (Healey 1980; Simenstad *et al.* 1982). Alternatively, Chum may pass directly through estuaries or remain for up to four weeks (Healey 1979; Levy and Northcote 1982; Simenstad *et al.* 1982). Although more variable, Coho also spend time rearing in estuaries (Miller and Sadro 2003) with residence times ranging from 6 to 40 days (Simenstad *et al.* 1982). Residence time for juvenile salmon can depend on habitat quality which can be assessed through measuring factors related to predator avoidance, the physiological adjustment from fresh to saltwater, migration habitats, and feeding/growth (Simenstad *et al.* 1982; Simenstad and Cordell 2000; Fresh 2006). Rapid growth during this time is critical for predator avoidance (Pearcy 1992) and to increase survival over a resource-limited winter (Beamish *et al.* 2004), therefore, juvenile salmon diet, and factors influencing it, are essential to study and will be the focus of chapter 2. Juvenile salmon are relatively opportunistic feeders and will consume a variety of different marine invertebrates and fish. Being similar in size, juvenile Coho and Chinook diets overlap and consist mainly of small crustaceans (e.g., amphipods and decapods) and insects, as well as fish as they grow larger and are less gape limited (Healey 1980; Brodeur and Pearcy 1990; Daly *et al.* 2009; Duffy *et al.* 2010). Generally, Chum enter the marine environment at a smaller size than Chinook and Coho and will feed on a different suite of smaller prey items such as copepods, smaller amphipods, and insects (Healey 1979; Brodeur and Pearcy 1990; Dumbauld *et al.* 2015). As such, modifications to coastal marine environments may impact salmon species differently depending on how their prey sources respond.

1.2. Anthropogenic modification to coastal environments

Humans have impacted coastlines worldwide resulting in pronounced ecosystem degradation (Lotze *et al.* 2006). A number of different factors contribute to degradation including eutrophication, climate change, pollution, overfishing, invasive species and habitat destruction or alteration (Kennish 2002). For the purposes of this thesis, I will be focusing on specific examples of habitat alteration (chapter 2) and pollution (chapter 3). The addition of infrastructure to coastal ecosystems has become prolific in many populated areas and yet the ecological ramifications associated with these specific modifications are relatively understudied (Bulleri 2005; Toft *et al.* 2007; Bulleri and Chapman 2010). Research indicates that recruitment, foraging, competition, predation and/or reproduction of marine organisms may be altered by the addition of artificial structures and suggests that these structures may not act as surrogates of natural habitat (Bulleri and Chapman 2010). The construction of docks and piers, for example, may decrease the habitat value for juvenile fishes (Able *et al.* 1999). In addition, shoreline armouring in nearshore ecosystems may disrupt terrestrial connectivity and impact beach wrack subsidies (Heerhartz *et al.* 2014), change invertebrate communities (Heerhartz *et al.* 2015), and impact juvenile salmon distribution, abundance, behaviour, and feeding (Toft *et al.* 2007, 2013; Morley *et al*. 2012; Munsch *et al*. 2015). Other shoreline structures such as boat ramps, bulkheads and jetties will also modify nearshore environments with potential ecological consequences (Kennish 2002; Bulleri and Chapman 2010). A large proportion of shoreline modification research has focused on shoreline armouring structures, however, we have relatively limited knowledge about other types of coastal alterations such as shellfish aquaculture which will be addressed in chapter 2.

1.3. Shellfish aquaculture and Baynes Sound

The shellfish aquaculture industry continues to develop and grow globally (FAO 2014) and is considered necessary to meet increasing populations and higher food demand (Costa-Pierce 2002). Canadian aquaculture was ranked 20th worldwide in 2009, with British Columbia (BC) generating over 60% of Canada’s production in 2011 (Nguyen and Williams 2013). Although less substantial than finfish culture, shellfish aquaculture also contributes significantly to the Canadian economy, particularly from Prince Edward Island and BC (Nguyen and Williams 2013).

For the purpose of this thesis, shellfish aquaculture will include bivalves only. Bivalve ground culture generally takes place in intertidal areas in protected bays with large mud or sand flats where the shellfish can be placed directly on the substrate (Simenstad and Fresh 1995). This type of culture is common for oysters, as well as clams that are placed higher in the intertidal zone. Ground culture involves the addition of structures such as intertidal fencing and antipredator netting (for clam culture) as well as the bivalves themselves. Through farming practices, Pacific oysters are often placed in the low intertidal zone on soft substrate which does not typically occur naturally as they prefer rocky habitats (Ruesink *et al.* 2005). Alternatively, oysters can be cultured in deeper nearshore waters using stake or hanging methods. Stake methods involve placing wooden posts in shallow areas that serve as attachment substrate for young oysters (spat) whereas hanging methods often involve floating rafts or suspended lines where longlines, trays or bags are hung to grow oysters (Baluyut 1989).

All types of bivalve culture require modification of intertidal and/or subtidal areas and thus a number of ecological changes have been observed. Cultured bivalves filter-feed on naturally occurring seston which can exert “top-down” impacts on phytoplankton in the water column (Newell 2004). This feeding can enhance water quality and the depth to which sunlight can reach, which in turn, increases the depth to which aquatic vegetation can grow successfully (Newell and Koch 2004) but could decrease the amount of food available to other filter feeders. Aquatic vegetation recovery and the many organisms that use these valuable habitats for rearing, feeding and refugia can benefit from these habitat improvements. However, filter-feeding by bivalves, particularly at high densities, can also increase the amount of feces and pseudofeces in the benthic environment (Hatcher *et al.* 1994). This influx of organic matter has been a common finding among shellfish aquaculture studies (Sorokin et al. 1999; Chamberlain et al. 2001; Christensen et al. 2003; Bendell-Young 2006). Increased amounts of organic matter and overall sedimentation can also be amplified by the addition of anti-predator nets (Simenstad *et al.* 1993; Spencer *et al.* 1997; Munroe and McKinley 2007) which are common aquaculture structures used to reduce predation on commercially valuable shellfish. In more extreme cases, it has been found that the increased sedimentation associated with shellfish aquaculture could lead to anoxic conditions and detrimental impacts to surrounding benthic communities (Sorokin *et al.* 1999; Bartoli *et al.* 2001; Beadman *et al.* 2004). In addition, with increased accumulation of sediment comes a greater likelihood that geochemical processes and nutrient cycling may be disrupted. Variations in nutrient dynamics appear to be dependent on local factors including type of shellfish aquaculture, bivalve densities and oceanographic conditions. In addition to nutrient dynamics and sedimentation, many studies have examined shellfish aquaculture's impact on species richness and diversity with varied results (Grant *et al.* 1995; Chamberlain *et al.* 2001; Crawford *et al.* 2003; Beadman *et al.* 2004; Bendell-Young 2006; da Costa and Nalesso 2006).

In BC, shellfish aquaculture occurs within juvenile Pacific salmon habitat providing the opportunity to examine interactions between this industry and this important fish species during a critical time in the salmon's lifecycle. Baynes Sound, located on the east coast of Vancouver Island, is one of the greatest shellfish aquaculture production sites in BC. It is also an ecologically and biologically significant area (EBSA) due to its uniqueness, with features such as thermally-stratified waters and large expanses of sheltered soft sediment (DFO 2013) which subsequently make it an ideal location to culture bivalves (Simenstad and Fresh 1995). In addition, Baynes Sound is home to large aggregations of overwintering birds, a prominent herring spawn and a number of Pacific salmon spawning streams that produce Coho, Chinook, Chum and Pink salmon (Jamieson *et al*. 2001).

In chapter 2, I explore the question of whether shellfish aquaculture modifications are influencing juvenile salmon abundance and diet. By looking at prey, abundance, feeding intensity and condition of juvenile Coho, Chinook and Chum salmon in shellfish aquaculture modified areas, I will investigate the implications of these changes and how these altered habitats may become more or less valuable. These results will contribute to the further understanding of factors related to early marine mortality in juvenile Pacific salmon and species-specific responses to shoreline modification.

1.4. Marine Pollution

In addition to shoreline modification, the shellfish aquaculture industry has been identified as a contributor to plastic debris in the marine environment through their abundant use of plastic materials in items such as ropes, netting, floats and trays. Approximately 18% of marine plastic debris is estimated to be produced from the fishing and aquaculture industries (Andrady 2011) and recent evidence has shown that the degradation of these plastics can produce microplastics (plastic particles < 5 mm) that fish can ingest (Wright *et al.* 2013). Throughout chapter 3, I explore marine plastic pollution and microplastics as another aspect of anthropogenic influence that may be affecting the early marine survival of juvenile salmon.

Plastic marine debris has become commonplace throughout the world's oceans (Derraik 2002). Of the approximately 275 million metric tons of plastic produced in 2010 (PlasticsEurope 2015), it is estimated that between 4.8 and 12.7 million metric tons entered the ocean (Jambeck *et al.* 2015). Production has continued to increase with 311 million metric tons of plastic produced globally in 2014 (PlasticsEurope 2015). Plastics are durable, lightweight and affordable (Laist 1987) which makes them ideal for many of their uses in society, however, these same properties are responsible for their persistence in the marine environment. The majority of plastics (80%) enter the ocean through land-based sources (Andrady 2011), however, fishing and aquaculture industries have been identified as additional contributors (Hinojosa and Thiel 2009; Mathalon and Hill 2014; Desforges *et al*. 2014). Derelict fishing gear can pose problems to marine organisms through ghost fishing as well as the breakdown into smaller particles or microplastics that can be ingested by marine life (Arthur *et al.* 2014).

Microplastics are found ubiquitously in the marine environment (Andrady 2011). Through anthropogenic pollution via primary sources (plastics manufactured at the micro scale) or secondary sources (the breakdown of larger plastics), microplastics have entered the marine environment on a global scale and are considered an emerging issue for the health and sustainability of our oceans (Derraik 2002; Barnes *et al.* 2009). Because of their small size, microplastics can be mistaken for prey items and have the potential to negatively impact marine organisms through ingestion (Cole *et al.* 2011; Wright *et al.* 2013). Microplastics have been quantified in a number of fish species (reviewed in

Lusher 2015) and negative impacts through laboratory studies have been observed (Rochman *et al.* 2013; Mazurais *et al.* 2015; Lonnstedt and Eklov 2016; Pedà *et al.* 2016) but it remains unclear whether these results are generalizable across different species and in a natural context. Though it is critical and timely to examine the health risks to fish associated with microplastic ingestion, it is important to examine these aspects at environmentally relevant concentrations for individual species in specific areas. In order to do this, baseline data must be collected and analyzed to quantify the incidence of microplastics in the organism and their marine environment. In chapter 3, I will address whether juvenile salmon are ingesting microplastics, the concentrations in their environment, and if this consumption may have implications for early marine survival.

1.5. Thesis goals

This chapter has provided an introduction to nearshore areas where juvenile salmon reside during a critical period of their lifecycle and the multitude of anthropogenic modifications and stressors they may experience. Habitat loss/ modification has long since been identified as one of the top threats to coastal ecosystems (Kennish 2002; Crain *et al.* 2009) and, while microplastic debris is a relatively new concern, early research suggests it should be considered an international priority (GESAMP 2015). The primary goals of this thesis are to investigate how environmental changes from shellfish aquaculture and plastic pollution may be affecting growth-determining factors of juvenile salmon associated with diet and ingestion. In chapter 2, I investigate the influence of shellfish aquaculture on juvenile Pacific salmon abundance and diet in Baynes Sound, BC and in chapter 3, I determine the incidence of microplastics in juvenile Chinook salmon in nearshore environments along the east coast of Vancouver Island. Chapter 4 summarizes and integrates the overall findings of chapters 2 and 3 and provides recommendations on how to move forward with regards to shellfish aquaculture, microplastics and juvenile salmon habitat conservation.

Chapter 2: The influence of shellfish aquaculture on juvenile Coho and Chinook abundance and diets in Baynes Sound, British Columbia

2.1. Introduction

Declines in Coho and Chinook salmon in the Strait of Georgia beginning in the 1970s have been attributed to over-fishing, habitat loss, and low marine survival (DFO 1991, 1999; Labelle *et al.* 1997; Beamish *et al.* 2008). Hatchery programs and stricter fishing regulations were implemented to enhance adult salmon populations (DFO 1991,1999) but despite this, escapements continued to decline to record lows. Estimates of total marine survival were closely linked to survival during juvenile salmon's first marine summer suggesting that early marine residence is a critical time to determine survivorship (Beamish and Mahnken 2001; Beamish *et al.* 2010). While the focus has been primarily on Chinook and Coho due to population declines, Chum salmon also experience similar pressures and have high early marine mortality (Healey 1982b).

Juvenile salmon early marine mortality

Juvenile Coho and Chinook salmon enter the marine environment after several months to two years in fresh water, whereas Chum begin their migration towards marine waters within days (Quinn 2005). When entering this new environment, juvenile salmon must adjust to a number of environmental changes (e.g., temperature and salinity) causing physiological stress (Healey 1980; Simenstad *et al.* 1982; Thorpe 1994) and mortality is typically high (Parker 1968). During this time, estuarine environments are important to juvenile salmon as they provide predator refuge and trophic resources (Simenstad *et al.* 1982; Thorpe 1994) which are key for foraging success leading to rapid growth. Therefore, the conditions (i.e., environmental variables, predation and food availability) that juvenile salmon experience when entering the marine environment may determine the survival outcome (Levings *et al.* 1986; Fresh 2006).

It is thought that this high mortality occurs during two stages. The first occurs upon initial entry into the estuarine or nearshore marine environment when juvenile salmon are vulnerable to high predation. Larger, faster growing juveniles generally have an advantage because of faster swimming speeds for predator avoidance (Pearcy 1992) and reduced vulnerability to gape-limited predators (Sogard 1997; Juanes *et al.* 2002). The second stage of high mortality occurs in late fall and winter when environmental conditions (e.g., food availability) are less ideal. Larger fish in higher condition are able to survive through periods of starvation and sub-optimal environmental conditions better than their smaller counterparts (Beamish *et al.* 2004). Juveniles must therefore grow to a large enough size by the end of their first marine summer to maximize their chances of survival over the following winter (Beamish and Mahnken 2001). Mortality over this resource-limited winter period may increase due to direct physiological reasons or indirectly from predation because of physiological limitations (Beamish *et al.* 2004). This critical size and period emphasizes the importance of juvenile salmon's first marine summer and the conditions they experience for growth and survival. Therefore, estuarine and nearshore habitats, where these juveniles develop and feed during their first marine summer, are extremely important as declines in early marine survival could be a result of poor feeding conditions (Duffy *et al.* 2010).

Anthropogenic modification and shellfish aquaculture

Juvenile salmon heavily rely on estuarine and nearshore areas during their early marine residence that often have been altered by human modifications. These changes can result in shifts of foraging behaviour and prey availability which may have negative consequences for juvenile salmon (Romanuk and Levings 2005; Toft *et al.* 2007; Munsch *et al.* 2015). Studies show higher Chinook survival in more pristine (i.e., less modified) estuaries (Magnusson and Hilborn 2003). Others have observed negative impacts on juvenile salmon prey availability due to shoreline armouring (Toft *et al.* 2007; Heerhartz and Toft 2015; Munsch *et al.* 2015), however, the ecological consequences of other forms of coastal modification are relatively understudied.

Shellfish aquaculture has resulted in intertidal habitat modification of many estuarine environments in the northeast Pacific (Emmett *et al.* 2000) but unlike shoreline armouring, shellfish aquaculture modifies the environment by adding infrastructure that may be increasing environmental complexity. Due to the ecological importance of various types of submerged aquatic vegetation, particularly for juvenile fish, comparisons have been made among shellfish aquaculture, eelgrass and mudflat areas. Generally, invertebrates are attracted to increased habitat complexity and aquaculture areas can act similarly to eelgrass in terms of supporting a diverse abundance of species (Hosack *et al.* 2006; Powers *et al.* 2007). Alternatively, fish such as juvenile salmon are more mobile and don't generally associate with specific benthic environments (Hosack *et al.* 2006; Dumbauld *et al.* 2015; Munsch *et al.* 2015) indicating that benthic modifications may not impact them directly. For example, Dumbauld *et al*. (2015) examined salmon diets across oyster culture tenures and undisturbed areas (mudflat, seagrass and channels) in a coastal estuary and found Coho, Chinook and Chum salmon diets were not strongly associated with the benthic environment, suggesting that modifications accompanying shellfish aquaculture may not have detrimental effects to this group of fishes. Thus far, a large portion of shellfish aquaculture studies has focused on the impacts on sediment and benthic communities underlying culture sites (Simenstad and Fresh 1995; Crawford *et al.* 2003; McKindsey *et al.* 2011) whereas a smaller portion have examined the impacts to higher trophic levels such as fish. Because of the expanding shellfish aquaculture industry, it is important to understand the ramifications of aquaculture development on ecologically important species such as juvenile Pacific salmon.

Baynes Sound

In BC, one of the greatest shellfish aquaculture production areas is Baynes Sound where approximately 50% of BC shellfish aquaculture is conducted (Truscott 2002). The Sound measures approximately 8700 hectares (Carswell *et al.* 2006) and, in addition to aquaculture, this area supports many important bird and marine species and is designated as an ecologically and biologically significant area (Jamieson and Levesque 2014). The most abundant and commonly cultured species in Baynes Sound are the Pacific oyster, *Crassostrea gigas*, and the Manila clam, *Venerupis philippinarum*, both of which were intentionally introduced from Japan in the early 1900s (Quayle 1964). A range of methods are used to culture these species including bottom culture in the intertidal zone and rafts in deep-water pelagic habitats.

Many "ecosystem services" are provided by shellfish including supply of valuable food resources and water filtration. However, shellfish practices involve landscape modification such as intertidal fencing, anti-predator nets, oyster rafts and outplanting which are not typical in natural intertidal environments. Shellfish aquaculture also adds a large amount of hard substrate in areas mainly consisting of soft substrate which, in turn, may support different types of habitat-forming algae. These changes in habitat complexity can alter the environment and the communities it supports. Despite the large-scale modifications of the intertidal and pelagic environments of Baynes Sound, we have yet to fully understand what these alterations mean for other organisms in the ecosystem, particularly at higher trophic levels.

Many rivers and creeks drain into Baynes Sound making it an ideal habitat and spawning ground for various fish species including ecologically and economically valuable Pacific salmon species (Jamieson *et al.* 2001). In addition, intertidal eelgrass beds act as nurseries and provide protection and valuable food sources for these salmon (Phillips 1984). Landscape modification for shellfish farming practices can damage eelgrass or, in more extreme cases, destroy entire beds (Simenstad and Fresh 1995). Understanding how changes to nearshore environments may impact ecologically important species such as Pacific salmon becomes increasingly important as natural habitats continue to decline.

Project objectives

Coastal development studies have largely focused on other types of shoreline development (e.g., shoreline armouring) and less is known about the impacts of modifications such as shellfish aquaculture on higher trophic level species such as fish. The objectives of this study are 1) to determine if juvenile Chinook, Coho and Chum salmon are using nearshore areas modified by shellfish aquaculture and investigate if there are differences in relative abundances at aquaculture and non-aquaculture sites and

2) to compare juvenile Chinook, Coho and Chum diets at aquaculture and non-aquaculture sites. By determining if and how Pacific salmon species are affected by shellfish aquaculture activities, we can assess how these species are responding to structures such as oysters beds, intertidal fencing, and anti-predator nets as nursery habitat during their early marine residence. These results will help us understand the consequences of coastal development and how it may affect other organisms as well as ecosystem services. In addition, this study will help determine factors related to early marine mortality in juvenile salmon and species-specific responses to shellfish aquaculture.

2.2. Methods

Study region

Baynes Sound is an ideal study region because of its extensive shellfish aquaculture presence and because it's a relatively small and enclosed study system. Three aquaculture and non-aquaculture paired sites were chosen throughout the Sound based on previously established sites with similar characteristics (Figure 1). Because a large portion of the shoreline is under active shellfish aquaculture tenure, control sites were challenging to locate and this limited the number of paired sites we could investigate. Sites were numbered from south to north with "A" referring to an aquaculture site and "NA" referring to the non-aquaculture reference sites. It must be noted that the "NA" sites may have had some bivalve culture in the past and/or undergo recreational harvesting, however, they are not currently active. Corresponding numbers refer to site pairings. Site NA1 is located on the Deep Bay Marine Field Station's research shellfish lease. It is in close proximity to the Deep Bay marina and has undergone some experimental small-scale shellfish ground culture in the past. The site, however, was never extensively farmed and was not active during our sampling season. The paired A1 site is located on an active shellfish tenure farming Manila clams and Pacific oysters via both ground culture and deep-water raft culture. Both A1 and NA1 were located in more sheltered areas whereas the other locations were more exposed to wind and wave action. Site A2 had similar characteristics to A1, hosting both clam and oyster ground culture, however, site A2 did not have deep-water culture using rafts. In the past, NA2 was an active shellfish culture area but has since been used for recreational harvest only and is not actively seeded. Site A3 is an active shellfish lease consisting of both clam and oyster ground culture. Sites A3 and NA3 were the smallest areas and had the most variable substrate types ranging from silt to larger cobble whereas the other sites primarily consisted of gravel and sand. Site A3 had both ground and deep-water culture. Sites A1, A2 and NA2 had relatively shallow slopes across larger areas while sites NA1, A3 and NA3 had steeper slopes across smaller intertidal areas.

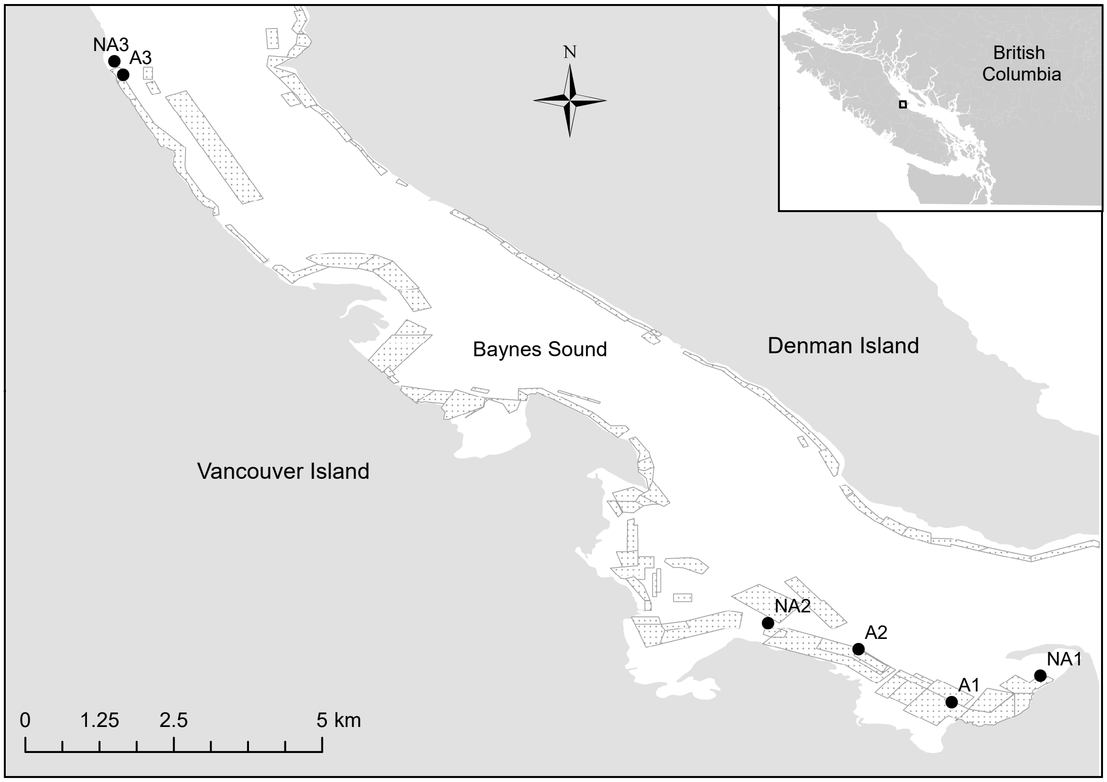

Figure 1: Study sites in Baynes Sound, BC where juvenile Chinook, Coho and Chum salmon were sampled for stomach content analysis. Three aquaculture (A) and three non-aquaculture (NA) sites were chosen throughout the Sound.

Sample Collection

Salmon

To determine relative abundance and collect a subsample of juvenile salmon for stomach content analysis, we beach seined at each site five times from May to July, 2014. In order to capture juvenile Coho, Chinook and Chum salmon, beach seining was completed using a 25 x 2 m seine consisting of 6 mm stretch mesh. During a low tide window (approximately one hour before and one hour after), two consecutive beach seine sets were completed along a span of 60 m (30 m per set) parallel to the shoreline below aquaculture areas (as to not interfere with aquaculture structures and bivalves). To begin a set, the seine was loaded into the bow of a 14-foot, 25-horsepower outboard aluminum boat. With one end of the seine anchored by a crew member on the beach, the boat would reverse in a semicircle formation piloted by a second crewmember, at which time a third crew member would disembark from the boat and haul the net towards the shoreline. Captured fish were corralled into the bunt of the beach seine and placed into a 1.2 x 1.2 m floating PVC square. This allowed quick sorting, identification and release of non-target species without removing them from their natural marine environment. Juvenile salmon were identified using characteristics in Hartman (1997), enumerated and a subsample of up to 20 per species per site were euthanized using an overdose of tricaine methanesulfonate (MS-222; 300 mg/L) and stored on ice (Animal Care Protocol # 2014-010(1), University of Victoria).

Environmental variables

At each sampling event, environmental variables including temperature, pH, dissolved oxygen (DO) and salinity were measured using pH and DO meters, and a refractometer. Site complexity was measured using a profile gauge adapted from McCormick (1994). During low tide, a 20 m wide area was measured from 1 to 3 m above mean lower low water (MLLW). The area was then assessed and divided based on prominent substrate type (e.g., boulders, cobble, shell). Throughout each area, three to five 0.5 x 0.5 m quadrats were randomly placed and three gauge measurements were taken in each quadrat. To do this, the gauge was gently placed in the quadrat, allowing the individual pegs to contour to the substrate. The gauge was levelled and then a photo was taken for later analysis. To account for vegetation that lies flat when air exposed but would provide structure when covered with water (e.g., eelgrass), we measured the height of any vegetation that came in contact with a peg. Using ImageJ software, the contour length (the length across the tops of all pegs) was measured. Any vegetation measurements were added to the peg height and incorporated into the contour length. From this, we compared the contour distance to the linear distance (0.5 m which was the straight line distance between the two outside pegs) and calculated a ratio to compare among sites. In each quadrat, we averaged three gauge measurements and then averaged all quadrats within the pre-determined area. Each substrate type was weighted depending on the proportion of area it covered in our study site and added together to produce an overall complexity estimate for each site. Other site characteristics such as most abundant intertidal and shoreline vegetation, freshwater influence, slope and anthropogenic influences in the surrounding area were recorded.

Stomach content analysis

Upon return to the laboratory, salmon identification was confirmed using features such as parr marks, anal fin shapes, and branchiostegal and pyloric caeca counts. Fish were measured to fork length (± 1 mm) and weighed (± 0.01 g). Stomachs were removed by making an incision from the anus to the operculum along the ventral surface of the fish, pulling out the intestine from the anus, clipping the oesophagus and removing the entire gut. The gut was placed in a 20 mL glass scintillation vial containing 10% buffered formalin until further processing.

Before examining contents, stomachs were removed from formalin, excess organs were removed and the stomach was blotted and weighed. The stomach was cut open and all contents were removed. The stomach lining was rinsed with deionized water and weighed to indirectly determine the weight of stomach contents alone. Under a dissecting microscope, prey items were identified at least to order. Once identified, prey groups were enumerated and weighed. Prey groups were also pooled by the habitat type from which they likely originated (i.e., benthic, planktonic, terrestrial). Prey items categorized as benthic included amphipods (Gammaridae, Caprellidae, Corophiidae), harpacticoid copepods, cumaceans, tanaids, isopods, polychaetes, ostracods, juvenile bivalves, and eggs. Planktonic prey items included fish, hyperiid amphipods, calanoid copepods, larval decapods (crabs and shrimp), nauplii, larvaceans, and cladocerans, and terrestrial prey items consisted of insects and collembolans.

Data analysis

Environmental data were compared among sites with repeated measures analysis of variance (ANOVA). Abundance, fish size, condition, feeding intensity and individual prey groups were compared among sites using ANOVA. In the case of a significant result, a Tukey-Kramer post-hoc test was employed to determine where the differences were. When data did not meet normality assumptions and could not be transformed, a Kruskal-Wallis test followed by Dunn's post hoc test were employed to determine individual site differences. Data were pooled by site type (i.e., aquaculture and non-aquaculture) if trends were consistent for all sites (i.e., data were consistently higher in one site type) and compared using Welch's t-test or a Wilcoxon rank sum test when data did not meet normality assumptions and could not be transformed. Fish abundance was measured with catch per unit effort (CPUE) consisting of the abundance captured at each sampling event. Condition was calculated using the equation:

$$K = \frac{W}{L^3}\ x\ 10^5$$

where K is the condition factor, W is the salmon wet body weight measured in grams and L is the fork length of the fish measured in millimeters (Meehan and Miller 1978).

Feeding intensity was determined using percent bodyweight (%BW) calculated by the equation:

$$\%BW = \frac{SCW}{BW \ - SCW} \ x \ 100$$

where BW is the salmon wet body weight measured in grams and SCW is the weight of the stomach contents in grams (Brodeur 1992).

Cumulative prey abundance curves were generated by plotting the cumulative number of prey items identified against the cumulative number of stomachs analyzed to ensure a representative amount of samples were analyzed. Stomach contents were analyzed using non-metric multidimensional scaling (nMDS) and permutational multivariate analysis of variance (PERMANOVA) in order to determine similarities and differences among sites, and between site types and species. All data were either square-root (Coho and chum prey abundance), or fourth-root (all other data) transformed to improve the representation of less abundant species and the resemblance matrices were generated using the Bray – Curtis coefficient. Empty stomachs were not included in stomach content analysis because there were relatively few (Coho: 1, Chinook: 2, Chum: 2) and obscured patterns. In addition, for Chum prey analysis, sites A3 and NA3 were not included as no Chum were captured at A3 and only one individual was captured at NA3. PERMANOVA designs were run for each data set (i.e., prey abundance and mass data sets for each salmon species) including site type as a fixed factor (two levels: aquaculture and non-aquaculture), site as a random factor nested within site type (six levels) and date as a random factor nested in site. When differences existed, similarity percentages (SIMPER) were used to determine which species were responsible for the dissimilarities. NMDS plots were produced to visualize each data set and were considered to be well representative of the data if stress <0.2 (Clarke 1993). Univariate statistics were completed using the statistical program R (R Core Team 2014) while multivariate analyses were completed using the ecological statistics program Primer (Clarke and Gorley 2015).

2.3. Results

Environmental measurements

There were no significant differences in any of the environmental measures across site type (Repeated measures ANOVA, $p > 0.05$) with the exception of a significantly higher dissolved oxygen measurement at site NA1 compared to A1 (Tukey post-hoc, $p = 0.032$). Complexity was not statistically significant between sites but there was a trend for higher complexity at all aquaculture sites compared to their non-aquaculture counterparts.

Salmon abundance

A total of 1046 Pacific salmon were captured consisting of three Pacific salmon species (Coho, Chinook and Chum). There were no significant effects of site type or site on mean salmon CPUE for any species (Kruskal-Wallis, $p > 0.05$). Overall our catch per unit effort (CPUE) of all species was 46.3 ± 71.8 (SD) at aquaculture sites and 9.6 ± 17.8 at non-aquaculture sites. Our CPUE was highest at site A2 (69.3 ± 81.1) followed by site A1 (65.8 ± 93.1) and NA1 (18.5 ± 31.0). NA2, NA3 and A3 had the lowest CPUE consisting of 5.5 ± 5.3, 4.8 ± 3.6 and 3.8 ± 4.1 at each site, respectively (Figure 2).

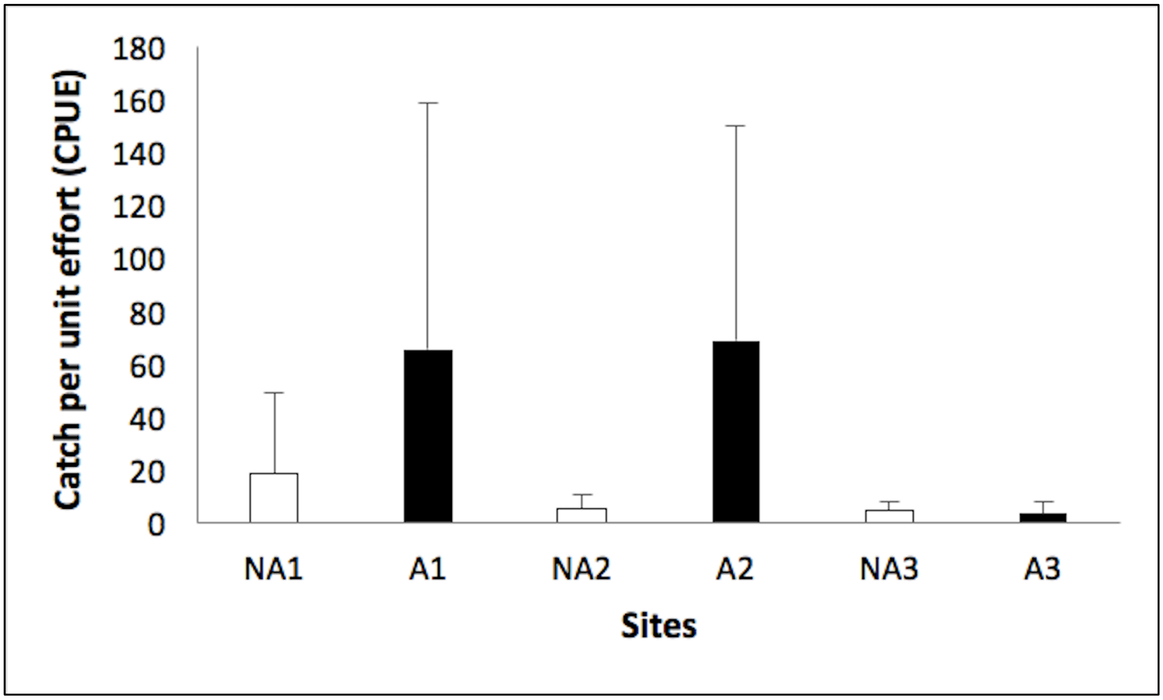

Figure 2: Juvenile Pacific salmon catch per unit effort (all species combined) across aquaculture (black bars) and non-aquaculture (white bars) sites in Baynes Sound, BC from May to July, 2014. Error bars represent the standard deviation.

Size and condition

Subsamples of 56 Chum, 100 Coho and 66 Chinook were euthanized for gut content analysis and size measurements. Mean fork length (± SD) for juvenile Chum salmon was 47.4 ± 10.6 mm, while Coho and Chinook were significantly longer at 98.3 ± 12.4 mm and 87.4 ± 10.7 mm, respecively (Dunn's test, $p < 0.05$). Coho were the heaviest at 11.6 ± 4.8 g and Chum were the lightest with an average weight of 1.13 ± 0.94 g with Chinook weighing 7.82 ± 3.10 g. All species weights were significantly different from one another (Dunn's test, $p < 0.05$). Coho had the highest condition factor (1.15 ± 0.10) followed by Chinook (1.12 ± 0.10) and Chum (0.86 ± 0.14) (Figure 3). Chinook and Coho condition were not statistically different, however, both were significantly higher than Chum (Tukey post-hoc, $p < 0.05$).

Site level differences existed for Coho weight and condition, and Chinook length (ANOVA, $p < 0.05$) and weight (Kruskal-Wallis, $p = 0.020$), but did not correspond to site type. There were no significant differences in Chinook condition factor or Coho length among sites (ANOVA, $p > 0.05$). Alternatively, Chum salmon were longer (Wilcoxon rank sum test, $p = 0.001$), heavier (Wilcoxon rank sum test, $p = 0.002$) and had a higher condition factor (Welch's t-test, $p = 0.017$) at aquaculture sites (Figure 3).

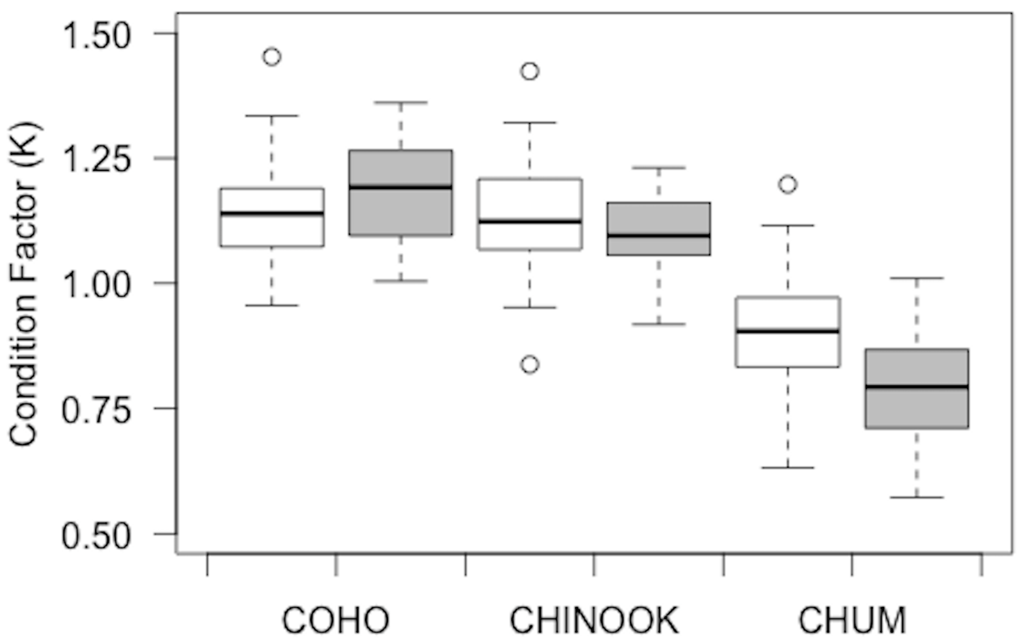

Figure 3: Condition factor (K) for Coho, Chinook and Chum over aquaculture (white) and non-aquaculture (gray) sites combined. Salmon were captured in intertidal areas across three aquaculture sites and three corresponding non-aquaculture sites. Boxes represent interquartile ranges with medians (black line) and the whiskers are minimum and maximum values. Open circles represent outliers.

Diet

Cumulative prey charts show the cumulative number of prey items identified plotted against the cumulative number of stomach samples. For all salmon species (Coho, Chinook, and Chum), the cumulative number of prey items steadily increased and then became asymptotic indicating that enough stomachs had been sampled to provide accurate representation of diet (Figure A9).

Coho

Coho ate a variety of prey items consisting primarily of insects (39.1%) and decapod crab larvae (29.6%) numerically (Figure 4), and fish (88.3%) gravimetrically. In addition, Coho ate decapod shrimp (3.0% by number, 0.71% by mass), copepods (6.5% by number, 0.05% by mass) and amphipods (4.3% by number, 1.4% by mass). Other less common prey items (making up 10.4% total) included collembolans, cumaceans, ostracods, and cladocerans. There were site level differences in feeding intensity (ANOVA, $p = 2.13e-05$), however these did not correspond to site type. Multivariate analysis of salmon prey also showed no significant differences in what Coho were ingesting at aquaculture and non-aquaculture sites (PERMANOVA, $p > 0.05$, Table 1,Figure 4). Non-metric multidimensional scaling (nMDS) supported these results and showed no sample grouping by site or site type (Figure 5). These results were consistent when examining prey numerically and gravimetrically. By number, the origin of Coho prey primarily consisted of planktonic prey (63.9%) and the remainder was terrestrial (23.0%), benthic (9.0%), and fish (4.1%) (Figure 6). By mass, Coho ingested predominantly fish (88.3%) followed by planktonic (6.6%), terrestrial (3.6%) and benthic prey (1.5%). The origin of Coho prey items was not significantly different between site types (Wilcoxon rank sum test, $p > 0.05$). Across all sites, Coho ingested more planktonic prey than benthic both numerically (Wilcoxon rank sum test, $p = 4.45E-05$) and gravimetrically (Wilcoxon rank sum test, $p = 0.001$). In addition, numerically Coho ate more planktonic than terrestrial and fish prey (Wilcoxon rank sum test, $p <0.05$). By mass, fish dominated over benthic, planktonic and terrestrial prey items (Wilcoxon rank sum test, $p < 0.05$). The nMDS showed no grouping by site type, however, samples moderately grouped by sample date and fish size class (Figure A10- A).

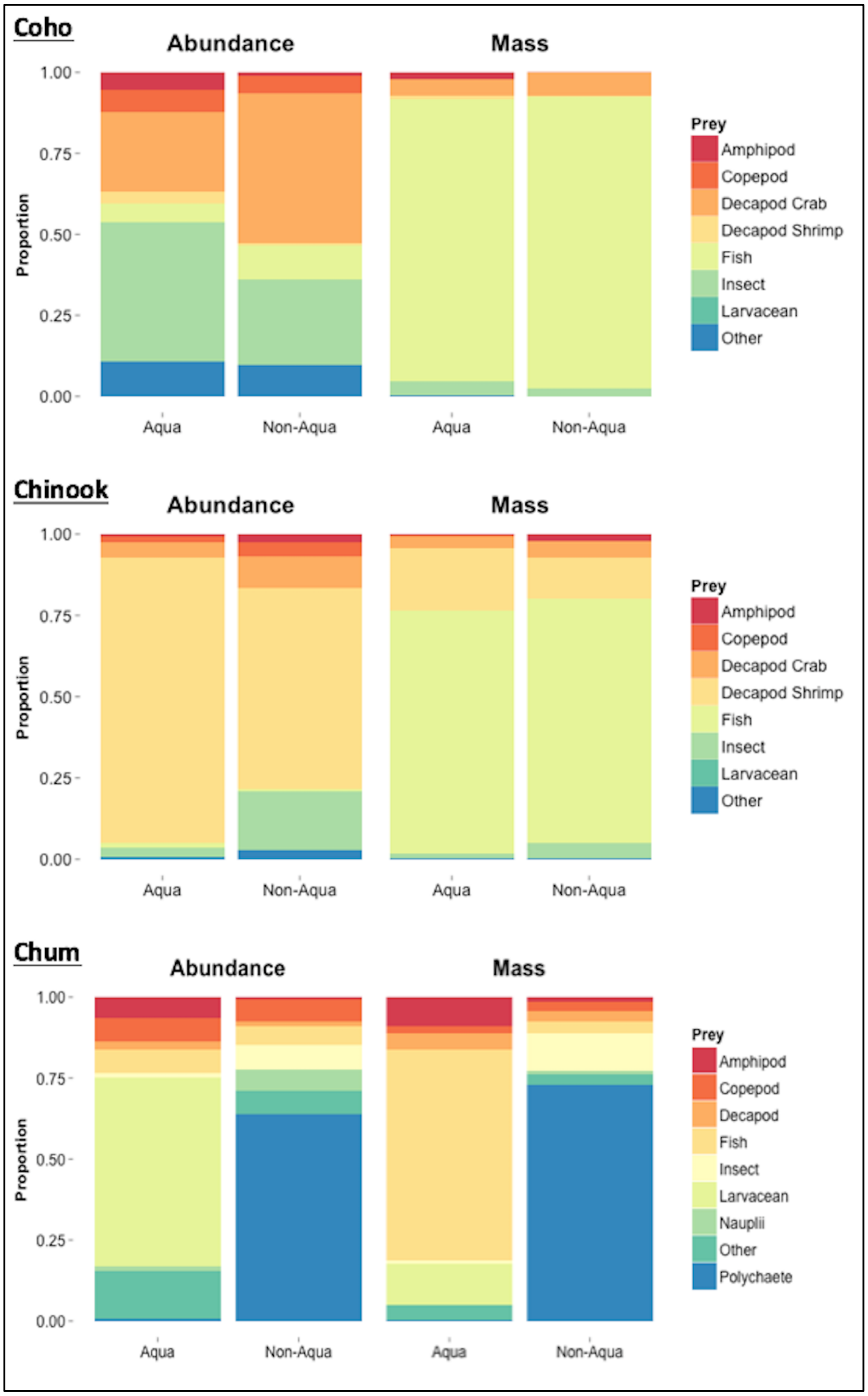

Figure 4: Stacked bar plots showing the numerical and gravimetric proportions of different prey items consumed by Coho, Chinook and Chum across aquaculture and non-aquaculture sites. Salmon were captured in intertidal areas across three aquaculture sites and three corresponding non-aquaculture sites.

Table 1: PERMANOVA results for Coho, Chinook and Chum prey captured across six sites in Baynes Sound, BC. ST = Site type (aquaculture and non-aquaculture), Si = Site (A1, A2, A3, NA1, NA2, NA3) and Da = sampling date.

Salmon Group Factor	df	SS	MS	Pseudo F	p
Coho, prey abundance					
ST	1	4447.3	4447.3	0.72469	0.576
Si(ST)	4	29318	7329.4	1.4764	0.190
Da(Si(ST))	8	60480	7560	3.8103	0.001
Residual	85	1.6865E+05	1984.1		
Total	98	2.8906E+05			
Coho, prey mass					
ST	1	3882.9	3882.9	0.6312	0.598
Si(ST)	4	30408	7602	1.6721	0.181
Da(Si(ST))	8	59679	7459.8	6.2117	0.001
Residual	85	1.0208E+05	1200.9		
Total	98	2.2147E+05			
Chinook, prey abundance					
ST	1	4660.3	4660.3	1.3013	0.290
Si(ST)	4	14616	3653.9	1.0291	0.408
Da(Si(ST))	8	29919	3739.8	1.382	0.038
Residual	50	1.35E+05	2706		
Total	63	1.92E+05			
Chinook, prey mass					
ST	1	4172.6	4172.6	1.1091	0.389
Si(ST)	4	15482	3870.4	1.0568	0.397
Da(Si(ST))	8	31439	3929.9	1.5914	0.016
Residual	50	1.23E+05	2469.4		
Total	63	1.84E+05			
Chum, prey abundance					
ST	1	11052	11052	1.6029	**0.001**
Si(ST)	2	13969	6984.4	1.4352	0.301
Da(Si(ST))	4	18081	4520.2	1.4648	0.043
Residual	43	1.33E+05	3085.9		
Total	50	1.78E+05			
Chum, prey mass					
ST	1	10769	10769	2.1879	**0.001**
Si(ST)	2	9939.1	4969.6	1.0854	0.478
Da(Si(ST))	4	17343	4335.7	1.4932	0.049
Residual	43	1.25E+05	2903.7		
Total	50	1.67E+05			

Chinook

Numerically Chinook diets were dominated by decapod shrimp (78.2%). In addition, Chinook consumed insects (8.5%), decapod crab larvae (6.6%), copepods (mostly harpacticoids; 2.8%), amphipods (1.3%), fish (1.1%) and a variety of other prey items in small amounts similar to those eaten by Coho (Figure 4). By mass, Chinook diets were dominated by fish (74.9%) and decapod shrimp (16.8%). There was higher consumption of insects at non-aquaculture sites both numerically (Wilcoxon rank sum test, $p = 0.025$) and gravimetrically (Wilcoxon rank sum test, $p = 0.024$). This trend was consistent among all sites except A3. There were no differences in other prey items between site type. In addition, there were site level differences in feeding intensity (Kruskal-Wallis, $p = 0.048$) but these were not consistent with site type. There was no sample grouping by site or site type (nMDS, Figure 5) which supported our results showing no significant differences in prey by site type (PERMANOVA, $p > 0.05$, Table 1). Numerically, Chinook diets consisted primarily of planktonic (45.3%) and terrestrial prey (37.2%), with benthic (12.6%) and fish (4.9%) making up the remainder (Figure 6). By mass, Chinook diets consisted mostly of fish (64.2%) with the rest consisting of planktonic (18.0%), benthic (15.6%) and terrestrial prey (2.2%). Between site type, juvenile Chinook ate more terrestrial prey items by number at non-aquaculture sites (Wilcoxon rank sum test, $p = 0.015$) and this trend was consistent for all sites except A3. Chinook also ate more terrestrial prey by mass at non-aquaculture sites (Wilcoxon rank sum test, $p = 0.016$) but this was not reflected in site specific differences. In general, Chinook also ate more planktonic prey items than benthic and fish numerically (Wilcoxon rank sum test, $p < 0.05$) and more planktonic prey items than terrestrial by mass (Wilcoxon rank sum test, $p = 0.004$). In addition, Chinook ate more terrestrial prey than fish (Wilcoxon rank sum test, $p = 0.024$). Unlike Coho, the nMDS showed no sample groupings for sample date and for fish size.

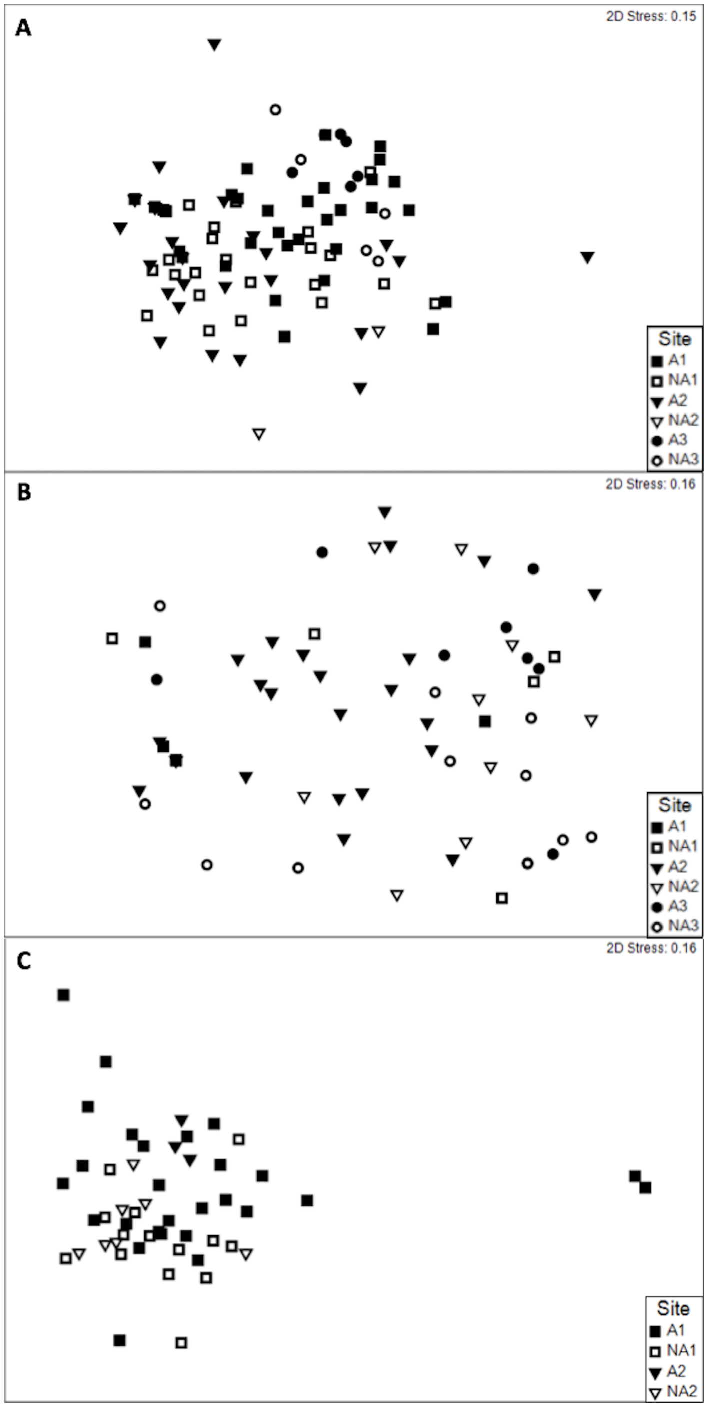

Figure 5: Non-metric multidimensional scaling ordination of prey abundance in Coho (A), Chinook (B) and Chum (C) salmon over aquaculture sites (black shapes) and non-aquaculture sites (white shapes). Data points that are closer together have more similar prey. Salmon were captured in intertidal areas across three aquaculture sites and three corresponding non-aquaculture sites.

Chum

Numerically Chum consumed large numbers of larvaceans (41.2%) and polychaetes (18.9%), although these were not consistent in all samples. Fish were the most abundant prey item by mass (56.3%) as well as larvaceans (11.5%) and polychaetes (8.1%). Other less common prey items (all < 10%) included amphipods (mostly gammarids), copepods (mostly harpacticoids), larval decapods, insects, collembolans, cladocerans, barnacle larvae and fish eggs (Figure 4). When examining chum diets across aquaculture and non-aquaculture sites, we found significantly more decapods (by abundance and mass) were consumed at aquaculture sites (Wilcoxon rank sum test, $p < 0.05$) whereas more insects (numerically) and polychaetes (numerically and by mass) were consumed at non-aquaculture sites (Wilcoxon rank sum test, $p < 0.05$). The nMDS plot displayed moderate grouping among samples from aquaculture and non-aquaculture site types (Figure 5). Despite site variation, Chum prey were significantly different between aquaculture and non-aquaculture site types (PERMANOVA, $p = 0.001$, Table 1). These differences were primarily caused by more insects, harpacticoid copepods, polychaetes and collembolans consumed at non-aquaculture sites and more fish eggs and larvaceans being consumed at aquaculture sites (SIMPER). Numerically, Chum diets consisted primarily of planktonic (55.2%) and benthic prey (39.8%). Terrestrial origin and fish prey made up the remaining 5% of the diet. By mass, fish made up the largest proportion of the diet (56.3%) with benthic (22.3%), planktonic (18.8%), and terrestrial (2.6%) making up the remaining proportions (Figure 6). Juvenile Chum salmon ate a higher proportion of planktonic prey by mass at aquaculture sites (Wilcoxon rank sum test, $p = 0.021$) while they ate significantly higher proportion of terrestrially derived prey at non-aquaculture sites (Wilcoxon rank sum test, $p = 0.041$). Although there were few differences in proportions of the various prey being ingested on aquaculture and non-aquaculture areas, feeding intensity (%BW) was significantly higher at aquaculture areas (Wilcoxon rank sum test, p = 0.009).

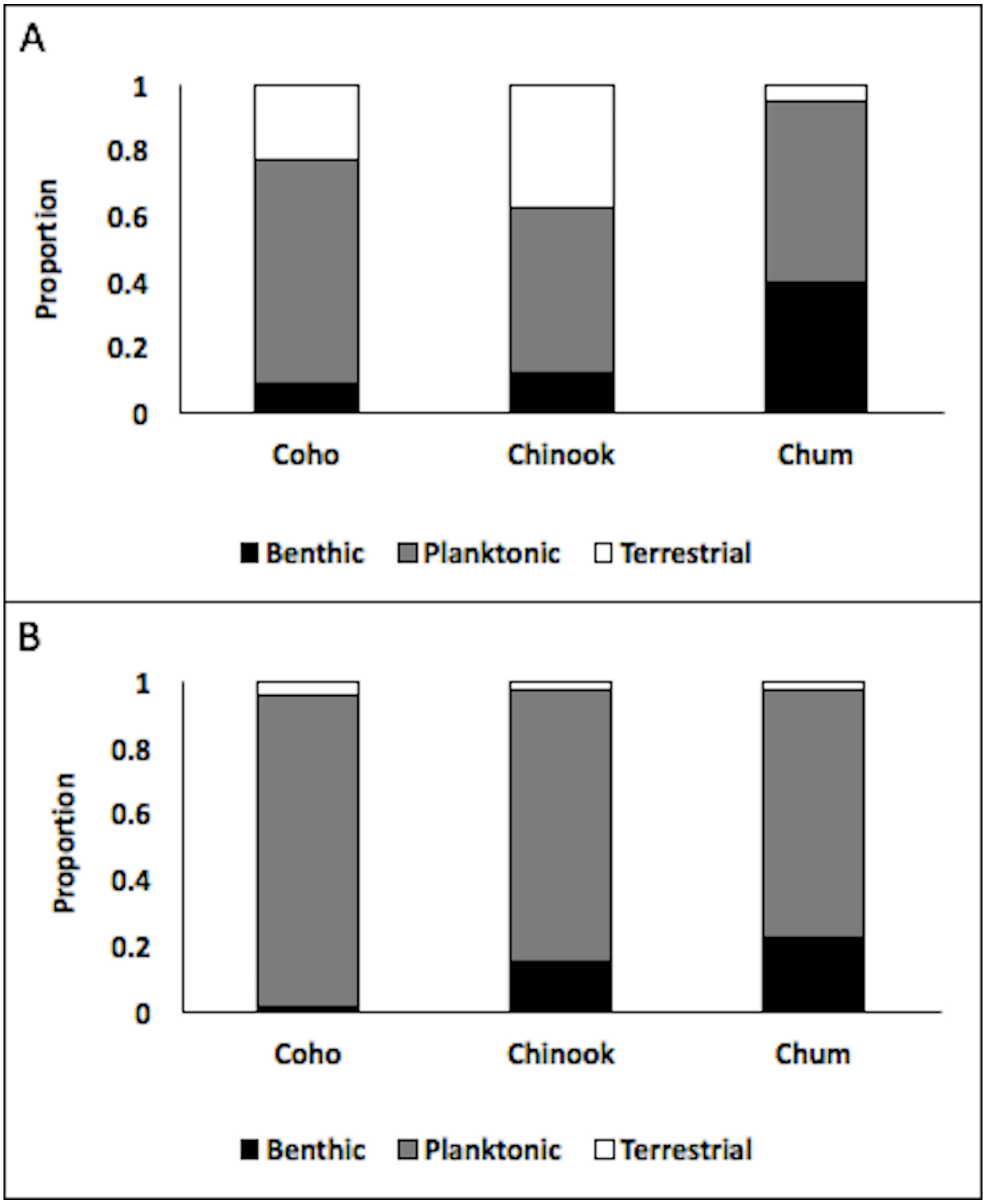

Figure 6: Species comparisons of proportion of prey origin by number (A) and mass (B) averaged across six sites in Baynes Sound, BC. See methods for prey species classification.

Species comparison

Coho and Chinook had more overlap of diets whereas Chum samples grouped apart from the other two species (Figure A10 - B). Coho and Chinook focused on larger prey items such as larval decapods and fish while Chum ate smaller prey such as larvaceans and small polychaetes. SIMPER analysis showed insects were important in all three species, while fish were important in Coho and Chinook diets, and collembolans, fish eggs and harpacticoid copepods were important to Chum. While Coho and Chinook had relatively similar diets, there were some marked differences. Coho ate a higher proportion of copepods (numerically) and fish (numerically and by mass; Wilcoxon rank sum test, $p < 0.05$) while Chinook ate a higher proportion of decapod shrimp (numerically and by mass; Wilcoxon rank sum test, $p < 0.05$). When examining prey origin, Chinook were ingesting more benthic and planktonic prey than Coho.

2.4. Discussion

Juvenile Coho, Chinook and Chum salmon, captured in Baynes Sound, BC, exhibited large variation in abundance and prey across the three aquaculture and three non-aquaculture sites sampled. As a species with a complex lifecycle, there are a number of important factors to consider when examining the abundance, condition and diet of juvenile Pacific salmon during early marine residency.

Abundance

In general, juvenile salmon abundance was highly variable among sites and site types. Although differences were not statistically significant, likely due to high variability in CPUE, we captured more juvenile salmon on aquaculture sites than non-aquaculture sites. Habitats with structure, like vegetation for example, can represent more desirable habitat due to higher food resources and refuge from predators (Heck *et al.* 2003; Mattila *et al.* 2008). In our case, the three-dimensional structure provided by the addition of high concentrations of bivalves in shellfish aquaculture locations may be acting functionally similar to aquatic vegetation. Indeed, studies have found that fish and invertebrates are attracted to the added habitat complexity provided by shellfish aquaculture (Dealteris *et al.* 2004; Powers *et al.* 2007). Dumbauld *et al*. (2015), however, found that juvenile salmon species (Coho, Chinook and Chum) exhibited no differential association among seagrass beds, oyster culture and mudflat areas within a Washington estuary. This is consistent with our results showing that, although there were more salmon captured over shellfish aquaculture areas, variation was high and results were not statistically significant. Differences in the number of juvenile salmon captured across individual sites likely result from site-specific characteristics and may be exclusive to species, life stages and varying types of modification (Dumbauld *et al.* 2015). Although not measured quantitatively in this study, differences may have resulted from varying degrees of vegetation cover and type and/or sediment type. Further, higher salmon abundances generally occurred in locations closer to spawning streams. Sites A1, NA1, A2 and NA2 are generally clustered in the southern portion of Baynes Sound where there are more spawning streams and higher salmon abundances whereas A3 and NA3 are further north towards the center of the Sound where spawning streams are less abundant and is perhaps why we captured fewer salmon at these sites. Further quantitative assessments on vegetation, substrate and proximity to spawning streams would be beneficial to this study.

Size and condition

Coho and Chinook were significantly larger than Chum. These differences are likely due to freshwater residence time as Chinook and Coho typically spend a longer period of time in freshwater (multiple months to 2 years) and thus emerge into the estuary at a larger size than Chum that will usually migrate to the marine environment within days of emerging from the gravel (Quinn 2005). Chum also exhibited a significantly lower condition factor than Coho and Chinook. Because Chum enter the marine environment when they are smaller they tend to feed on smaller, less energy-rich prey relative to high quality prey items such as fish which were more important in Coho and Chinook diets (Beauchamp 2009; Duffy *et al.* 2010). This ontogenetic shift to piscivory that is typically observed in juvenile salmon (Daly *et al.* 2009; Duffy *et al.* 2010) may have led to the higher Chinook and Coho condition factors compared to Chum. In addition, Chinook and Coho tend to grow faster than Chum salmon (Healey 1980) which allows them to shift to larger lipid-rich prey earlier in their nearshore residence. Hamilton *et al*. (2002), who conducted juvenile salmonid surveys througout the Courtenay River estuary and northern Baynes Sound in January to August, 2001, observed the same patterns in condition factor with Coho and Chinook having similar condition factors with both being significantly higher than Chum.

We found no consistent significant differences in condition between aquaculture sites and non-aquaculture sites for Chinook and Coho. Alternatively, Chum had a significantly higher condition factor at aquaculture sites. Chum were found to ingest significantly higher amounts of decapods at aquaculture sites, however, because decapods contributed less than 5% both numerically and gravimetrically to Chum diets, it is unlikely this is the cause. Chum had a significantly higher feeding intensity (%BW) at aquaculture sites indicating that, although the proportions of prey ingested were similar between site types, the absolute mass of prey ingested per gram of fish was higher at aquaculture sites. This result may be due to differences in environmental availability of prey which were not examined here, however, a number of studies have found higher abundances of epibenthic invertebrates (i.e., chum prey items) associated with shellfish aquaculture (Hosack *et al.* 2006; Powers *et al.* 2007; Kelly *et al.* 2008). Alternatively, because of their smaller size and close association with the benthic environment, Chum may benefit from the different type of complexity offered by shellfish aquaculture structures. For example, although Chum consumed similar diets across both aquaculture and non-aquaculture sites, areas with lower complexity may be considered more "risky" with more effort and energy expenditure taking place for predator avoidance (Biro *et al.* 2003) and prey capture (Giacomini *et al.* 2013).

Comparing diets across species

Coho diets were dominated numerically by insects and decapods (mostly megalops crab larvae) which is consistent with other studies in the northeast Pacific that also found insects (Brodeur 1989) and megalops crab larvae to be important constituents of juvenile Coho diets in nearshore areas (Brodeur and Pearcy 1990; Daly *et al*. 2009). Likewise, in Chinook, insects and decapods (mostly shrimp however) made up large numerical proportions of the diet. Chum diets had high proportions of larvaceans which is consistent with the results of Dumbauld *et al*. (2015). Unlike Coho and Chinook, insects did not represent a large portion of Chum diets. Insects represent a high quality prey item (Duffy *et al.* 2010) and emphasize the importance of the intertidal connection to terrestrial habitats. Interestingly, Toft *et al*. (2007) examined juvenile salmon abundance and behaviour along anthropogenically modified shorelines and found that terrestrial prey are reduced in areas with shoreline armouring and overwater structures. Although insect prey was found in higher proportions in diets of Chum and Chinook from non-aquaculture sites, there were no significant differences in insects in Coho diets between site types. This suggests that shellfish aquaculture may not represent the same types of insect-limiting modifications as shoreline armouring and/or salmon species may respond differently to such modifications. Additional changes to insect availability may be determined by differences in freshwater input (Duffy *et al.* 2010) or amount and proximity of riparian influence surrounding the sites (Simenstad *et al.* 1982) which perhaps explained why fish from sites A3 and NA3 (closest proximity to riparian areas) had the highest terrestrial influence.

Results of the nMDS indicated that Coho diet was more associated with sample date and fish size whereas Chinook and Chum diet did not appear to show any groupings. This suggests that Coho are more opportunistic foragers than Chum and Chinook and adjust their diet as prey fluctuate spatially and seasonally as their size increases which in turn would allow for access to different prey items. These results are consistent with Daly *et al*. (2009) who also found Coho to be more opportunistic and Chinook to be more selective. Coho ingested a significantly higher proportion of fish than Chinook, contrary to other studies (Brodeur and Pearcy 1990; Schabetsberger *et al*. 2003; Brodeur *et al*. 2007; Baldwin *et al*. 2008). Our results are likely a product of fish size as Coho were larger, and thus less gape limited, and further along in their ontogenetic diet shift towards piscivory. Dumbauld *et al*. (2015) also found that Coho consumed more fish in their study and determined that because Chinook were smaller than Coho at the point of capture, piscivory was less important during that life stage.

Chinook and Coho consumed a higher proportion of planktonic prey items than benthic items both numerically and gravimetrically. However, shellfish aquaculture primarily modifies the benthic environment perhaps explaining the lack of diet differences across site types for these species. In contrast, Chum consumed more benthic prey items relative to Coho and Chinook, perhaps explaining why we observed differences in prey between aquaculture and non-aquaculture areas. Despite this however, there were no significant differences in the proportion of benthic prey items consumed by juvenile Chum between site types, however, differences in prey at non-aquaculture (more insects, harpacticoid copepods, polychaetes and collembolans) and aquaculture (more fish eggs and larvaceans) sites were observed. Furthermore, Chum had a higher feeding intensity over aquaculture sites suggesting that prey availability (for Chum specifically) may be higher at aquaculture sites. These results are consistent with Morley *et al*. (2012) and Munsch *et al*. (2015) who found shoreline modification (armouring) altered juvenile Chum diet, likely due to changes in epibenthic prey items, and had no effect on juvenile Chinook salmon that fed primarily on planktonic prey items and were relatively more mobile across larger landscapes. These results, however, also contrast with ours because the shoreline modification in their case was suggested as having negative consequences whereas shoreline modification in our study appears to be benefiting Chum salmon.

Overall, stomach content analysis of juvenile salmon sampled across aquaculture and non-aquaculture sites indicated that species feeding on more pelagic prey items (i.e., Chinook and Coho) were relatively unaffected by the habitat modifications associated with shellfish aquaculture. Alternatively, species that had a larger proportion of benthic prey relative to other species, were more associated with the benthic environment and therefore are more likely to show differences in diet. Chum had a higher condition factor across aquaculture sites suggesting the prey items associated with these sites may have been more abundant and higher quality and/or the increased/different type of complexity associated with shellfish aquaculture sites may have provided Chum with a higher quality habitat in which they could thrive. However further study is needed as there are likely many other factors at play aside from diet that may influence condition and overall effects on species fitness and survival could not be determined.

Future studies should examine environmental prey availability in these aquaculture areas to determine which prey items (if any) are being impacted by benthic modifications and how this relates to important prey items for juvenile salmon. In addition, increased sampling throughout the day would improve the holistic view of juvenile salmon diet. Estuaries and nearshore areas represent important habitat for juvenile salmon during a critical time in their lifecycle and modifications to these areas can cause species-specific responses. These results emphasize the importance of understanding the consequences of changing natural habitats for all species utilizing these environments.

Chapter 3: Microplastics in juvenile Chinook salmon and their nearshore environments

3.1. Introduction

Juvenile Chinook salmon face many challenges upon entering the marine environment including change in diet, predators, and physiological changes associated with the adjustment to higher salinity water (Healey 1980; Simenstad *et al.* 1982; Thorpe 1994). During this time, juvenile salmon are met with a number of anthropogenic pressures including modified habitats and pollution (Jackson *et al.* 2001; Lotze *et al.* 2006; Bulleri and Chapman 2010). Chapter 2 addressed habitat modification in the form of shellfish aquaculture, and pollution will be the focus of this chapter. Marine pollution includes the anthropogenic input of different substances in the marine environment including excessive nutrients, organic carbon and toxic chemicals (Kennish 2002). Plastic pollution specifically has been identified as a substantial threat to marine ecosystems (Derraik 2002; Kennish 2002) including both macro (>5 mm) and microplastics (<5 mm) (Andrady 2011). While we know more about other types of marine pollution, less is known about the emerging threat of microplastics.

Microplastics can be produced from primary or secondary sources. Primary sources include plastics produced at the micro scale such as microbeads in facial cleaners and toothpaste (Fendall and Sewell 2009). Along with microplastic particles produced from washing synthetic clothing (Browne *et al.* 2011), microbeads are transported to the marine environment through wastewater outfall (Carr *et al.* 2016). In addition, macroplastics from litter and industry materials (e.g., derelict fishing gear) can breakdown into smaller particles and eventually into microplastics (Arthur *et al.* 2014).

Microplastics have been found in most ecosystems spanning the globe including remote areas such as Arctic ice (Zarfl and Matthies 2010) and deep sea sediments (Van Cauwenberghe *et al*. 2013). The ingestion of microplastic particles has been documented in a variety of marine species ranging from zooplankton to fish to marine mammals with potential risks including internal physical damage (e.g., abrasion or blockage) and/or chemical harm (Cole *et al.* 2011; Wright *et al.* 2013). Microplastics can have concentrations of persistent organic pollutants (POPs) several orders of magnitude higher than ambient marine waters (Mato *et al.* 2001) and thus ingestion by lower trophic level organisms can provide an entry point for POPs to amplify through the food chain (Teuten *et al.* 2009). Microplastics, and negative impacts associated with their consumption, may pose a risk to humans (Choy and Drazen 2013; VanCauwenberghe and Janssen 2014; Rochman *et al.* 2015; Vethaak and Leslie 2016) and thus, essential marine organisms for human food security (e.g., fish and bivalves) are a priority in microplastics research (GESAMP 2015).

The incidence of microplastics has been documented globally in a large range of fish species (reviewed in Lusher 2015). Because microplastics are found in both water and sediment (GESAMP 2015), both benthic and pelagic feeders have the potential to ingest microplastics through direct consumption or by ingesting prey containing microplastics (Setälä *et al.* 2014; Desforges *et al.* 2015). Studies have yet to look at microplastics in juvenile Pacific salmon but have observed variable plastic abundances in many other fish species (reviewed by Lusher 2015). Despite the number of studies that have observed the occurrence of microplastics in fish, relatively few have examined how/if the presence of these particles impacts organismal health.

Microplastics have several negative impacts on fish (Rochman *et al.* 2013; Lonnstedt and Eklov 2016; Pedà *et al.* 2016). The extent and type of impact appears to depend on exposure concentration, plastic type, size and shape, as well as associated chemicals. In addition, responses differ among species and size (Wright *et al.* 2013) with early life stages potentially being more vulnerable (Lonnstedt and Eklov 2016). Effects on fish include intestinal alteration (Pedà *et al.* 2016), liver toxicity (Rochman *et al.* 2013), changes to hatching, growth, feeding preferences and predator avoidance (Lonnstedt and Eklov 2016), and increased mortality (Rochman *et al.* 2013; Mazurais *et al.* 2015; Lonnstedt and Eklov 2016; Pedà *et al.* 2016).

Coastal marine ecosystems have higher concentrations of microplastic pollution compared to their offshore counterparts (Desforges *et al.* 2014) and a large source of plastic pollution is believed to originate from anthropogenic land-based operations (Barnes *et al.* 2009; Browne *et al.* 2010, 2011; Doyle *et al.* 2011), therefore, nearshore environments, and the organisms that reside there, may be among the most directly and highly affected. Juvenile Chinook salmon depend on nearshore areas extensively in order to feed and grow (Healey 1980; Levy and Northcote 1982; Thorpe 1994) and thus early marine residency and any significant factors affecting survival success during this time must be examined carefully.

The objectives of this study were to determine 1) the microplastic concentrations in juvenile Chinook salmon across several nearshore areas along the east coast of Vancouver Island, 2) environmental (water and sediment) microplastic concentrations, and 3) relationships between environmental and organismal concentrations. Project results will indicate if, and to what extent, microplastics pose a threat to juvenile salmon.

3.2. Methods

Study sites

This project took place along the east coast of Vancouver Island bordering the Strait of Georgia (Figure 7). Sites were chosen based on previous research, in the case of Deep Bay (see chapter 2 study), and sites near freshwater tributaries containing prominent Chinook salmon runs. We chose four locations including Deep Bay, Big Qualicum, Nanaimo harbour, and Cowichan Bay. The Deep Bay site was located in the southern portion of Baynes Sound and was chosen based on successful juvenile Chinook capture during previous studies (see chapter 2 study). This site was located on an active shellfish aquaculture tenure consisting of both intertidal and deep-water culture and may contain Chinook salmon from the Puntledge River (both wild and hatchery) in addition to the Rosewall Creek hatchery (Hamilton *et al.* 2008). The Big Qualicum River is located 17 km north of Parksville and connects Horne Lake to the Strait of Georgia. It contains a wild Chinook run as well as inputs from the Big Qualicum hatchery. The Nanaimo River estuary is located in south Nanaimo and is bordered by downtown Nanaimo on the western shore and Duke Point to the east. It consists of an extensive tidal mud flat and hosts juvenile Chinook originating from the Nanaimo River (Healey 1980) as well as the Nanaimo hatchery. Cowichan Bay receives inputs from both the Cowichan and Koksilah rivers and is one of the biggest estuaries in BC (Argue *et al.* 1986). It contains inputs from both the Cowichan River hatchery as well as naturally occurring wild runs.

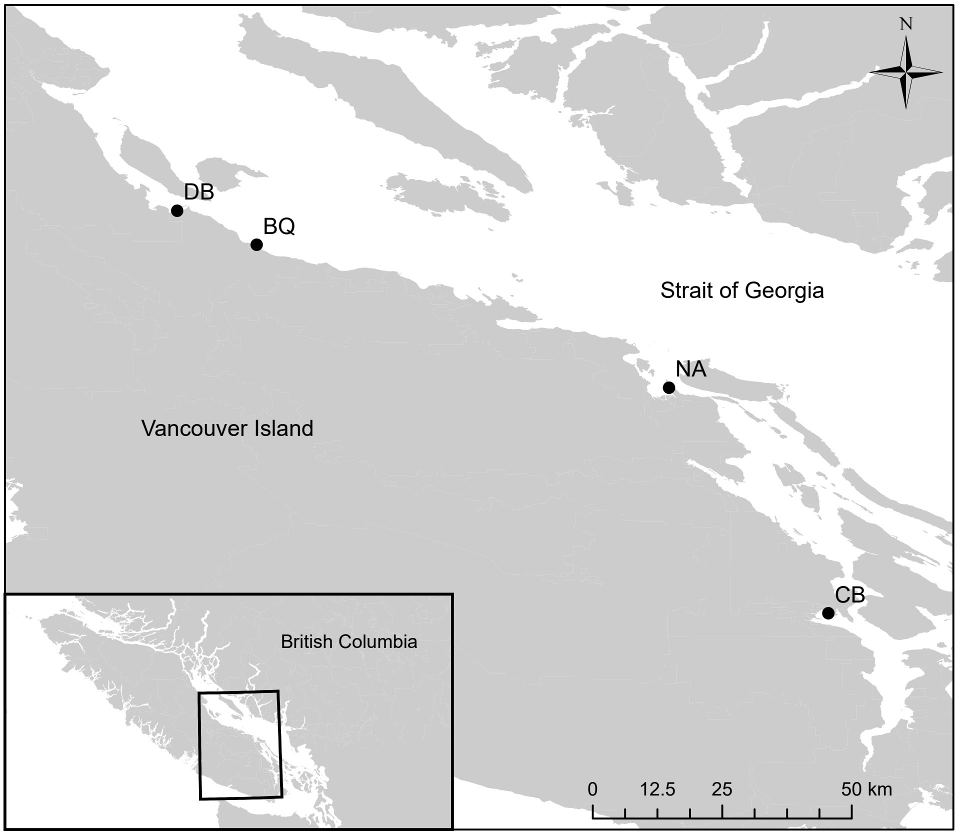

Figure 7: Study sites along the east coast of Vancouver Island where juvenile salmon, water and sediment were sampled for microplastics presence. DB = Deep Bay, BQ = Big Qualicum, NA = Nanaimo, CB = Cowichan Bay.

Sample collection

Juvenile Chinook

We used a 15 x 2 m seine consisting of 6 mm stretch mesh to capture juvenile Chinook salmon during June and July, 2015 at each of the four pre-selected sites. Beach seines were conducted at low tide windows between 0.5 and 1.1 m for Deep Bay, Big Qualicum and Cowichan Bay sites depending on beach accessibility. Nanaimo was an exception because it was accessible at high tides only, therefore sampling was conducted during 2.8 to 3.7 m tides. Between 3 and 8 beach seine sets were completed at each site until at least 40 fish were captured or catch per seine was below one.

The beach seine was set using an outboard aluminum boat. Captured fish were corralled into the bunt of the beach seine and placed into a 1.2 x 0.6 m floating PVC rectangle. This allowed quick sorting, identification and release of non-target species without removing them from their natural environment. Juvenile Chinook were identified using characteristics in Hartman (1997), enumerated and euthanized (up to a maximum of 40 per site) using an overdose of MS-222 (300 mg/L) and stored on ice (Animal Care Protocol # 2015-018(1), University of Victoria).

Water

We collected 25 L of surface water during low tide at each site spanning approximately 100 m (~25 m between samples) of shoreline where fish sampling took place. We filtered the water samples using a 100 um plankton net with a 30 cm diameter opening. Contents were rinsed and concentrated into the codend then transferred into sample containers. GPS locations were recorded and samples were stored at -12°C.

Sediment

Sediment samples were taken at the mid tidal zone (1.5 to 2.2 m) using a PVC sediment corer. Eight samples of 15 cm depth by 4 cm diameter were taken approximately one meter apart and stored in Ziploc bags. GPS locations were recorded and samples were stored at -12°C.

Microplastic extraction

Methodology testing

Microplastics research is still in its infancy and there are no standardized methods for plastic extraction for organic tissue, water or sediment. We tested various methods that have been presented in the literature including oxidation, acid, and basic digestion, as well as various density separation protocols. The following protocols are a culmination of the most successful methodologies for microplastic extraction from juvenile Chinook, intertidal water and sediment samples.

Juvenile Chinook

Upon return to the laboratory, fish were scanned for pit tags and their identification was confirmed using external features such as parr marks and anal fin shapes, in addition to branchiostegal and pyloric caeca counts. Fish were measured for fork length (± 1 mm) and weighed (± 0.01 g). Stomachs were removed by making an incision from the anus to the operculum along the ventral surface of the fish, pulling out the intestine from the anus, clipping the esophagus, and removing the entire gut. The gut was stored in a 20 mL glass scintillation vial containing 70% ethanol until further processing. During microplastic extraction, scintillation vials containing fish guts were covered with 100 um mesh and dried at 60°C overnight (or until sample was completely dry) in a conventional laboratory drying oven. Once dry, samples were crushed using the blunt end of a stainless steel probe and 10-15 mL of 10% filtered potassium hydroxide (KOH) was added to the vial in order to digest organic material. Samples were re-covered with 100 um mesh and returned to the drying oven and allowed to digest for 24 hours at 60°C. Following organic matter digestion by KOH, samples were vacuum filtered over a

Whatman GF/C 1.2 um glass microfiber filter paper. Resulting filter papers were stored in sealed petri dishes until microscopic examination.

Water

Water samples were subsampled in 50 mL portions, placed into 500 mL glass mason jars, and covered with 100 um mesh secured by the mason jar screw top. Moisture was removed through the mesh by inverting the mason jar over a filter flask attached to a vacuum pump. Three times the sample volume (~100 – 200 mL) of KOH was added to each mason jar and placed in the drying oven for 24 hours at 60°C in order to digest organic material. After 24 hours, KOH was removed with the vacuum filter (as above) and the sample was rinsed with filtered deionized water (FDI) to remove any excess KOH. Samples were centrifuged with FDI at 3500 g for 10 minutes in 50 mL centrifuge tubes and supernatant was poured off into a FDI rinsed flask. Samples were then centrifuged with filtered saturated calcium chloride ($CaCl_2$: density: 1.38 - 1.40 g mL^{-1}). This process was repeated a total of three times, pouring off supernatant and shaking vigorously to break apart the concentrated sample pellet each time. Combined supernatants were then vacuum filtered over Whatman GF/C 1.2 um glass microfiber filter paper. Resulting filter papers were stored in sealed petri dishes until microscopic examination.

Sediment

Sediment samples were dried in tin foil covered foil pans for 48-72 hours in a conventional drying oven at 70°C. Samples were homogenized using a ceramic pestle and approximately 70-80 g of sediment was sieved through a 4.75 mm and 90 um mesh, 7.5 cm diameter stainless steel sieve set. Sediment grains larger than 4.75 mm and smaller than 90 um was discarded. Sediment was transferred to a 500 mL glass mason jar and covered with 100 um mesh secured by a mason jar screw top. Excess moisture was removed through the mesh by inverting the mason jar over a filter flask attached to a vacuum pump and samples were dried for 24-48 hours. Dried sediment weight was determined indirectly by weighing the mason jar and dried sediment then weighing the empty jar following the extraction process. Approximately 100 – 200 mL of 10% filtered KOH was added to the mason jars containing the dried sediment and placed in the drying oven at 60°C for 24 hours to remove organic matter. KOH was removed from samples using the vacuum pump as above and rinsed three times with FDI. Sediment was transferred into 250 mL volumetric flasks and each sample was partitioned into five 50 mL centrifuge tubes in approximately 10 g increments. Samples were then centrifuged and filtered as per water samples.

Microplastic enumeration

The process of microplastic enumeration was identical for all sample types. Filter papers with samples were placed on a FDI dampened gridded glass petri dish and examined under a dissecting microscope. Using 40 – 50x magnification, filter papers were systematically examined for microplastic particles. Under this magnification, characteristics such as bendability, colour and twistedness were used for initial microplastic assessment. Potential microplastics were mounted on glass slides and examined under a compound microscope. At the higher magnification, characteristics such as structure and colour were used to further confirm microplastic assessment. Confirmed microplastics were enumerated and classified by colour and type (fibre, fragment, film or pellet).

Contamination

A number of steps were taken to eliminate plastic contamination of our samples. Any solution used during microplastic extraction was made using deionized water and filtered over a Whatman GF/C 1.2 um glass microfiber filter paper. Non-plastic equipment was used as much as possible and all glassware and equipment was washed thoroughly with soap and water followed by a FDI wash consisting of three separate rinses. Immediately following FDI rinses, glassware and equipment were covered with tin foil to protect from airborne plastic contamination. Equipment such as forceps were rinsed with FDI immediately before each use and samples were kept covered as much as possible throughout processing. When transferring samples between different vessels, thorough rinses (x3) were done to ensure complete transfer of material. All work was completed in a laminar flow hood and laboratory workers wore natural fibre clothing and 100% cotton laboratory coats in the laboratory at all times during microplastic extraction processing. At least three procedural blanks were run in conjunction with each sample batch to determine the amount and types of contamination present in our samples. Plastic contamination was then averaged across each set of blanks by colour and subtracted from each sample in the corresponding batch.

Data analysis

Microplastic concentrations were compared among sites using a one-way analysis of variance (ANOVA). In the case of water and sediment samples, microplastics concentrations were square root transformed to meet normality assumptions. When differences existed among sites, a Tukey post-hoc test was used to assess which sites were significantly different. Fish microplastics data were not normal after transformation and so a Kruskal-Wallis rank sum test was performed to determine differences among sites. Spearman's rank correlation was used to determine relationships between juvenile Chinook and environmental (water and sediment) microplastic concentration levels. The number of plastic particles in fish was converted to microplastics g^{-1} to standardize across fish sizes while plastics in water samples were converted to microplastics m^{-3} and sediment plastics were expressed as microplastics kg^{-1} dry weight (d.w.).

3.3. Results

In all sample types the majority (>90%) of microplastics were fibrous in nature as opposed to fragments, films or pellets and therefore all results will refer to fibres only. All plastics found were 100-5000 um in length and approximately 10-20 um in diameter. The codend we used to filter water had a 100 um mesh, therefore, this was deemed the lower size limit of plastics in water, fish and sediment samples.

Juvenile Chinook microplastic concentrations

We extracted microplastics from a total of 74 juvenile Chinook salmon (fork length: 78.2 ± 9.3 mm SD; wet weight: 6.01 ± 2.33 g) across four different sites along the east coast of Vancouver Island including Deep Bay (*n*=19), Big Qualicum (*n*=9), Nanaimo (*n*=7) and Cowichan Bay (*n*=39). Of the juvenile salmon sampled, 59% contained at least one plastic particle with a mean of 1.15 ± 1.41 microplastics per individual (0.24 ± 0.36 microplastics g^{-1}) across all sites. Fibrous microplastics made up 95% of plastic particles identified in juvenile Chinook salmon. There were no significant differences among the number of microplastics g^{-1} of juvenile Chinook sampled across our four sample sites (Kruskal-Wallis $p = 0.490$; Figure 8A). The most abundant fibres by colour were clear (41%) and blue (20%) for all sites.

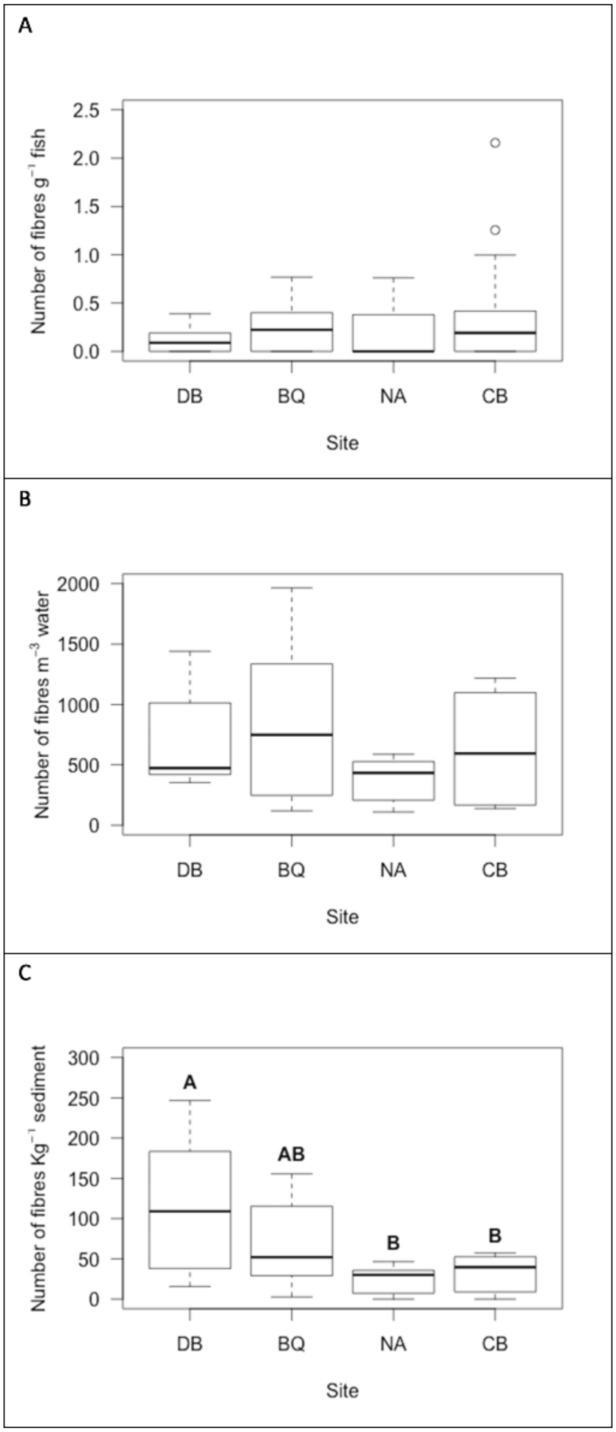

Figure 8: Average number of plastic fibres g^{-1} of fish (A), fibres m^{-3} of water (B) and fibres Kg^{-1} of sediment (C) across samples sites including Deep Bay (DB), Big Qualicum (BQ), Nanaimo (NA) and Cowichan Bay (CB). Boxes represent interquartile ranges with medians (black line) and the whiskers are minimum and maximum values. Open circles represent outliers and different letters represent significant differences among sites.

Water microplastic concentrations

Across all samples, we found a mean of 659.88 ± 520.87 microplastics m^{-3} with 100% of the samples containing at least one microplastic particle. As in fish, the majority of plastic particles found (93%) were fibrous plastics. There were no significant differences in the average number of plastic fibres m^{-3} in water samples across our four sample sites (ANOVA $p = 0.571$; Figure 8B). Similar to the juvenile Chinook samples, clear (70%) and blue (12%) fibres were the most abundant across all sites.

Sediment microplastic concentrations

Across all sites we found a mean of 60.2 ± 63.4 microplastics kg^{-1} d.w. of sediment sampled with 87.5% of samples containing at least one microplastic particle. The majority (91%) of microplastic particles identified were fibres. We found significant differences in microplastic concentrations among sites (ANOVA $p = 0.015$; Figure 8C). Deep Bay had significantly more microplastics than Nanaimo (Tukey post-hoc, $p = 0.020$) and Cowichan Bay (Tukey post-hoc, $p = 0.045$), while Big Qualicum was not significantly different from any other site (Tukey post-hoc, $p > 0.05$). By colour, clear was the most frequently identified microplastic fibre (64%) while blue (15%) was the next most common across all sites.

Correlations

There were no significant relationships between juvenile Chinook and environmental microplastic concentrations ($p>0.05$) nor was there a significant relationship between the environmental (water and sediment) levels ($p>0.05$).

3.4. Discussion

Microplastics are an emerging threat to marine ecosystems (Andrady 2011) and juvenile Chinook salmon may be at risk during a critical time in their lifecycle. In this study, juvenile Chinook salmon, water and sediment samples were examined to determine microplastic abundances in Chinook and their coastal environments. Relatively low concentrations of plastic fibres in fish samples which were not correlated with water and sediment microplastic concentrations.

Microplastic in juvenile Chinook salmon

We sampled 74 juvenile Chinook across four different sites and found that 59% contained at least one plastic fibre. Although previous studies have shown relatively high variance in the proportion of fish containing microplastic particles (range: 0.03% - 67%; reviewed in Lusher 2015), our results suggest a relatively high proportion of fish with at least one plastic particle in their gastrointestinal tracts. The consequences of microplastic ingestion are poorly understood and depend on type, shape and size of plastics. Mazurais *et al*. (2015) and Pedà *et al*. (2016) both conducted laboratory studies with European sea bass (*Dicentrarchus labrax)* and each tested a different type of plastic pellet (polyethylene and polyvinylchloride, respectively). Both studies found significant health consequences (e.g., intestinal alterations, mortality), however, the type, extent and severity varied substantially. In addition to differences in plastic characteristics, studies examining microplastic ingestion in fish also look at a wide variety of fish species and life stages. Lonnstedt and Eklov (2016) examined European perch (*Perca fluviatilis*) larvae in a laboratory study and found impacts to hatching, growth, feeding preferences and predator avoidance. If juvenile salmon experience similar impacts, their survival may be affected greatly as they experience high predations rates (Parker 1968) and growth is critical during this early marine life stage (Beamish and Mahnken 2001).

The range of plastics found per individual fish in this study (1.15 ± 1.41) is within the ranges observed previously of 0.33 – 7.2 and a mean of 1.78 in a variety of different sized fish species (Lusher 2015). In a study of microplastic particle ingestion by zooplankton in the northeast Pacific Ocean, Desforges *et al*. (2015) estimated that juvenile salmon in coastal BC could consume 2 – 7 microplastic particles per day. Mazurais *et al.* (2015) observed that a high proportion of polyethylene beads were being egested by sea bass larvae within 48 hours therefore other species such as salmon may do the same. If so, our microplastic quantities observed in our juvenile Chinook are likely underestimations of total microplastic ingestion.

Despite high egestion rates, Mazurais *et al.* (2015) determined that a plastic loading of 3.3 ± 0.19 polyethylene beads in larval European sea bass caused significantly higher mortality than the control group. Given the size difference in fish between the sea bass study (~ 2 – 12 mg) and the current study (6010 ± 2330 mg), we could predict that the same plastic loadings would have a less significant impact on our juvenile Chinook and that, although species-specific responses may differ between sea bass and salmonids, it is likely that the average microplastic concentration of 1.15 ± 1.41 found in our study is not causing significant mortality events.

Microplastic in water samples

Across all water samples, we found a mean of 659.88 ± 520.87 microplastics m^{-3}. Desforges *et al*. (2014) estimated a mean of 3210 ± 628 microplastics m^{-3} in the Strait of Georgia which is higher than those found in this study, however, differences in sampling methodologies (i.e., looking at a larger size range: 62.5 um – 5 mm) may partially account for their higher estimates. Desforges *et al*. (2014) also found that plastic particle size increased with distance from shore, indicating that the smaller size fractions may be more abundant in nearshore coastal sites like ours. Although our concentrations were low compared to Desforges *et al*. (2014), we found relatively high concentrations compared to studies from other coastal regions worldwide that, for the most part, observed <1 microplastic m^{-3} (reviewed in Lusher 2015). As most studies used a mesh size of 333 um (GESAMP 2015), however, results are likely underestimations for reasons outlined above. These findings emphasize a need for standardization and the use of smaller mesh sizes to accurately assess microplastic concentrations worldwide. Differences in microplastic concentrations could also be influenced by weather (Moore *et al.* 2002; Lattin *et al.* 2004). Storm events, particularly in the winter, were found to increase microplastic concentrations via mixing throughout the water column (Doyle *et al.* 2011) and runoff from land-based sources (Moore *et al.* 2002). Because our study was conducted during late spring/early summer (when juvenile salmon were utilising the nearshore areas), and stormy weather was rare, our results may differ from those conducted during other seasons.

Microplastic in sediment samples

Across all sites, we found a mean of 60.2 ± 63.4 microplastics kg^{-1} d.w. of sediment sampled. Cluzard *et al*. (2015) sampled sediment from various sites throughout Baynes Sound and found the median number of microplastics kg^{-1} d.w. to range from 0 to 85. In the region that included a sample area in a similar location to our Deep Bay site, a median of 24.6 microplastics kg^{-1} d.w. was documented which was lower than the mean of 115.40 microplastics kg^{-1} d.w. found in our study. This inconsistency may be due to differences in plastic extraction techniques (i.e., NaCl flotation) which may lead to underestimations of higher density plastics on their part. In addition, their study only looked at plastic particles larger than 1 mm in size which would also account for the higher concentrations found in our study as we included plastics from 100 um to 5 mm in size. Microplastic concentrations in Cluzard *et al.* (2015) and our study were also consistent with many European coastal studies (Thompson *et al.* 2004; Browne *et al.* 2011; Claessens *et al.* 2011) but lower than Mathalon and Hill (2014) who documented microplastic ranges of 200 – 800 microplastics kg^{-1} in sediment from Halifax Harbour, Nova Scotia. Mathalon and Hill (2014), however, did not correct their values despite high laboratory contamination levels and therefore, microplastic concentrations are likely overestimations. Sediment microplastic loads will also be affected by plastic type (e.g., PVC is a higher density plastic that will tend to sink in lower density salt water more readily than lower density plastics such as polyethylene; Van Cauwenberghe *et al*. 2015), geography and oceanography (Vianello *et al.* 2013), sediment type (Strand *et al.* 2013), and amount of environmental biofouling (Thompson *et al.* 2004; Barnes *et al.* 2009; Browne *et al.* 2010).

Site comparisons

Among sites, we only found differences in sediment microplastics concentrations. Because fish and water move throughout ecosystems, microplastics may disseminate far from their original source. Alternatively, sediment is more likely to remain relatively stationary, and may retain microplastics more readily. In other words, water and fish samples may represent a more instantaneous microplastic loading versus a more integrated picture of microplastics within the sediment (Davis and Murphy 2015).

Significantly higher microplastic abundances were observed in sediments at Deep Bay compared to the Nanaimo and Cowichan sites. The Deep Bay site is located in a sheltered bay and has a relatively shallow slope while both the Nanaimo and Cowichan Bay sites are located in more exposed areas with steeper slopes. Various studies suggest that exposed areas with higher wave energy may accumulate less microplastics and the enclosed geometry of harbours, for example, may trap microplastics and increase benthic settlement (Claessens *et al.* 2011; Vianello *et al.* 2013; Cluzard *et al.* 2015). Therefore, differences in site morphology may result in higher microplastic retention in sites like Deep Bay where plastic would likely settle more readily. Claessens *et al*. (2011) speculated that harbour activities (e.g., recreational boating) added to the microplastic load which is also consistent with our study as the Deep Bay site is within close proximity to the Deep Bay Marina. Furthermore, sites with freshwater inputs generally have higher microplastics loads (Claessens *et al.* 2011; Vianello *et al.* 2013) and although all of our sites had freshwater inputs, the Deep Bay and Big Qualicum sites, which had the highest sediment microplastic concentrations, were in the closest proximity to freshwater tributaries with Chef Creek and Big Qualicum River, respectively, emptying directly into the sites. The Nanaimo and Cowichan Rivers may also influence microplastic loads in their associated sites but to a lesser extent as they were further away (~2 km).

In addition, the Deep Bay sample site is located on a shellfish aquaculture tenure which may have direct plastic inputs through the high use of plastic netting, ropes, trays, and expanded polystyrene floats. Although the other sites have a range of other potential plastic sources, none are in as close proximity to a source as the Deep Bay site. Aquaculture has been identified as a potential microplastics source (Hinojosa and Thiel 2009; Cole *et al.* 2011; Mathalon and Hill 2014; Desforges *et al.* 2014) and cannot be discounted, however, these higher concentrations may also be linked to site oceanography and bathymetry.

Environmental microplastic availability

We found no significant correlations between the amount of microplastics found in water or sediment samples with the amount ingested by juvenile Chinook indicating that environmental levels do not necessarily reflect the amount ingested by marine organisms in this area. Desforges *et al*. (2015) compared microplastic abundance in zooplankton to water samples and found abundances were not correlated based on absolute number of plastic particles ingested, however, they suggested that levels must be corrected for zooplankton density because with a higher abundance of individuals, there may be less plastics available in the environment. Once the correction was made, higher ingestion rates corresponded with higher microplastic concentrations in water. Employing a similar correction may not be appropriate for fish as they are larger and more mobile than zooplankton and thus the lack of correlation was expected.

We also found that water microplastic concentrations do not correlate with sediment concentrations from the same sites. This may reflect differences in low density (floating) and high density (sinking) plastics across each site or differential biofouling that may be occurring causing low density plastics, that would normally float, to sink (Thompson *et al.* 2004; Barnes *et al.* 2009; Browne *et al.* 2010). Additionally, plastic sinking rates may be altered through ingestion by zooplankton followed by egestion via faecal pellets (Cole *et al.* 2016). This disparity among water, sediment, and fish samples may also be a product of the patchy distribution of plastics in the marine environment (Moreira *et al.* 2015) demonstrated by the relatively high standard deviations across all three types of samples. Future studies should attempt to collect larger sample sizes to increase the power of the findings.

Microplastics can originate from a number of different sources and through a combination of oceanography, wind and marine organisms, the transfer and dispersal of plastic particles can be extensive. Because of this complexity, linking microplastics to a specific source becomes challenging. Deductions can be made, however, based on various plastic characteristics. For example, we found that the majority of microplastics found in juvenile Chinook, water and sediment samples were fibrous in nature as opposed to being a film, fragment or pellet. Desforges *et al*. (2014) found a negative relationship between the proportion of fibres in the plastic samples and distance from shore indicating that nearshore areas will have generally higher fibre content than offshore areas. Moreover, they estimated that samples from areas we examined would have greater than 80% fibre content which is consistent with our findings. A significant source of fibrous microplastics originate from textiles and through sewage output with up to 1900 fibres per garment per wash being released from household washing machines (Browne *et al.* 2011). Large cities such as Vancouver, Victoria (Desforges *et al.* 2014) and Nanaimo, among others, are in close proximity to our sampling sites, releasing sewage effluent into the Strait of Georgia and potentially delivering a constant source of microplastics to the marine environment. Given the dominance of fibrous plastics, other fibre sources close to our sample areas include the extensive use of synthetic fishing line, nets and rope in fishing and aquaculture industries (Claessens *et al.* 2011).

On a larger scale, it has been demonstrated there is a strong relationship between the abundance of marine debris and increased human population (Barnes 2005). Although not quantified statistically, environmental or organismal plastic concentrations did not reflect population size as Nanaimo had a considerably larger population and either lower or no differences in plastic concentrations compared with the other sites. This is consistent with Claessens *et al*. (2011) who also did not find a correlation between human presence and microplastics and instead suggested that concentrations are more associated with the geography of the sample location. This idea is likely more applicable to regional studies (e.g., the current study) rather than studies at global scales.

Conclusions

This study examined the incidence of microplastic particles in juvenile Chinook and their nearshore environment along the east coast of Vancouver Island. We found predominantly microplastic fibres in the majority of our samples supporting the notion that microplastics are widespread and ubiquitous in the marine environment. We did not find significant differences in microplastic abundances ingested by juvenile Chinook or in water samples among our sample sites but found significantly more plastic fibres in sediment at our Deep Bay site likely due to geographical differences in oceanography and potential source inputs (e.g., shellfish aquaculture, marina, recreational boating) in the surrounding area. Our results demonstrate that environmental concentrations (i.e., water and sediment) do not necessarily reflect the amount of plastics found in juvenile Chinook salmon but this may be confounded by a number of factors including zooplankton and fish density which this study did not measure. These results also show that plastic concentrations vary within habitat types in the environment likely because sediment may accumulate higher microplastic abundance due to its relatively stationary nature.

Our microplastic abundances in salmon were low based on existing knowledge and seem unlikely to cause negative impacts, however, further studies on plastic retention time and health implications in juvenile Pacific salmon are necessary to confirm this. Studies examining the ingestion pathway (direct vs. indirect) and bioaccumulation would also benefit this field of study by improving environmental and organismal microplastic predictability. Microplastic fragments in surface water continues to rise as global plastic production increases (Barnes *et al.* 2009) indicating that although microplastic particles may not be an immediate threat to juvenile Chinook salmon, the future is uncertain. As there is little information on bioaccumulation of plastics and their pollutants, it is unclear how whole ecosystems may be affected and what the implications are for human health (Vethaak and Leslie 2016). With increased use of plastics in today’s society, this will continue to be a problem and there remains many knowledge gaps to fill in order to accurately assess the risk.

Chapter 4: Conclusions

Nearshore ecosystems are an important habitat for juvenile salmon during a life stage that will likely determine their overall survival. An understanding of all factors potentially related to increased mortality during this time is essential. We investigated two different anthropogenic stressors in nearshore environments including modifications associated with intertidal shellfish aquaculture and plastic pollution.

In chapter 2, I investigated the influence of shellfish aquaculture structures on juvenile salmon and determined that juvenile Coho and Chinook diet was relatively unaffected by the presence of shellfish aquaculture, whereas prey consumed were significantly different between aquaculture and non-aquaculture areas in Chum salmon. These results were likely the product of Chum salmon's higher reliance on the benthic environment for foraging and refugia relative to Coho and Chinook. In addition, Chum condition was significantly higher at shellfish aquaculture sites compared to non-aquaculture sites which may be due to higher prey abundance and/or habitat quality at aquaculture sites. All salmon species had variable diets and Coho in particular was likely a more opportunistic forager. This feeding plasticity may be a beneficial attribute (Munsch *et al.* 2015) as habitats continue to be driven away from their historical baselines (Lotze *et al.* 2006). For example, our study area Baynes Sound has persisted through other industries and development including substantial logging, mining, hydroelectric dams, agriculture and urban development (Hamilton *et al.* 2008). Despite numerous historical perturbations, Baynes Sound has demonstrated remarkable resilience and is currently designated an ecologically and biologically significant area (Jamieson and Levesque 2014). Research using similar sampling sites to ours in Baynes Sound found no difference in intertidal fish abundance, diversity and functional diversity between aquaculture and non-aquaculture sites (Bourdon 2015). Although juvenile salmon were not included in the analysis, this supports the notion that shellfish aquaculture in this area is not degrading habitat value for fish species in general. In addition, Bourdon (2015) observed that the fish communities were highly functionally redundant, suggesting that despite significant shellfish aquaculture, and other historical modifications in Baynes Sound, the ecosystem continues to be resilient to disturbances. These results are consistent with our study that showed negligible to positive effects of shellfish aquaculture on juvenile salmon abundance, condition and diet. However, our knowledge of the short and long term repercussions of modifications to these essential coastal ecosystems is still lacking and we don't fully understand the broader ecological consequences (Bulleri and Chapman 2010). Future studies would benefit from examining juvenile salmon (particularly Coho and Chinook) diet over larger spatial scales (e.g., Vancouver Island) rather than small regions (e.g., Baynes Sound) because of their high mobility and use of diverse habitats (Dumbauld *et al.* 2015; Munsch *et al.* 2015). For example, Baynes Sound as a whole could be designated as a "highly altered/modified" area to be compared with more pristine/natural regions. In addition, future work should address impacts to other species using these areas and also determine what these changes mean for long-term fitness. Overall, this study demonstrated that shellfish aquaculture infrastructure may have similar habitat value to less modified habitats and can support juvenile salmon. Although, we don't know if juvenile salmon's use of these nearshore environments has decreased over the timescales in which we've seen salmon declines or over which aquaculture has increased, currently, juvenile salmon appear to be using shellfish aquaculture as functional nursery habitat during their nearshore residence, suggesting that these modifications may not significantly impact early marine survival. However, although we did not observe negative repercussions to juvenile salmon in Baynes Sound specifically, the expansion and management of this industry should be approached with caution until we have a better understanding of cumulative ecosystem level effects and consider all different types of stressors.

In chapter three, we examined the incidence of microplastics in juvenile Chinook salmon along the east coast of Vancouver Island and found salmon in this area primarily ingest fibrous microplastics in low to average concentrations relative to those observed in other coastal regions (Lusher 2015). Microplastics extracted from Chinook and water samples were not significantly different across all sites sampled along the east coast of Vancouver Island, however, our Deep Bay site contained significantly higher sediment microplastic concentrations. High sediment concentrations were likely the product of site characteristics. Deep Bay was more sheltered and the sediments were more likely to retain microplastics compared to the other sites. In addition, this site was located on a shellfish aquaculture lease and near a marina putting it close to several potential microplastic sources (e.g., nets, ropes, trays, floats).

These results suggest that microplastic pollution in this area is not an immediate threat to juvenile Chinook salmon however, with continued increase of plastic production (PlasticsEurope 2015) and use, it is unlikely this problem will subside. As food fish, juvenile salmon are particularly important to monitor because of their connection to human consumption and potential health implications (GESAMP 2015; Vethaak and Leslie 2016). In addition, salmon play important roles in many food webs and through plastic and contaminant bioaccumulation there is potential for ecosystem level effects (Desforges *et al.* 2015). Because microplastics research is in its infancy, many knowledge gaps exist including the identification of sources, transport processes (Solomon and Palanisami 2016), and microplastic sample collection and extraction methodologies (Ivar do Sul and Costa 2014). Despite these gaps, current research strongly suggests that microplastics are an issue for ocean health and sustainability and this should be sufficient evidence for policies to be implemented (Rochman *et al.* 2016). Ultimately, the general public must become more informed and this issue needs to be addressed at multiple levels (i.e., local, regional and global) in order to produce change (Solomon and Palanisami 2016).

This thesis has addressed the importance of nearshore areas to juvenile Pacific salmon and a number of anthropogenic stressors they have adapted to and continue to face during their early marine residence. In comparison with other anthropogenic stressors such as global climate change and complete habitat destruction, as well as the prospect of cumulative effects, shellfish aquaculture modifications seem less imminent and globally threatening. The outlook for the emerging issue of microplastics, however, is continuing to decline as we complete more research, and a great amount of work must be completed in the near future to understand and mitigate this escalating threat. Despite the multitude of unanswered questions relating to factors contributing to early marine mortality of juvenile Pacific salmon, it is evident that nearshore areas provide essential habitat during a critical time and must be conserved in ways that ensure juvenile salmon can thrive and return in the future.

Bibliography

Able, K.W. (2005) A re-examination of fish estuarine dependence: Evidence for connectivity between estuarine and ocean habitats. *Estuarine, Coastal and Shelf Science* **64**, 5–17.

Able, K.W., Manderson, J.P. and Studholme, A.L. (1999) Habitat quality for shallow water fishes in an urban estuary: The effects of man-made structures on growth. *Marine Ecology Progress Series* **187**, 227–235.

Andrady, A.L. (2011) Microplastics in the marine environment. *Marine Pollution Bulletin* **62**, 1596–1605.

Argue, A.W., Hillaby, B. and Shepard, C.D. (1986) Distribution, timing, change in size, and stomach contents of juvenile chinook and coho salmon caught in Cowichan estuary and bay, 1973, 1975, 1976. *Canadian Technical Report of Fisheries and Aquatic Sciences*, 170.

Arthur, C., Sutton-Grier, A.E., Murphy, P. and Bamford, H. (2014) Out of sight but not out of mind: Harmful effects of derelict traps in selected U.S. coastal waters. *Marine Pollution Bulletin* **86**, 19–28.

Baldwin, R.E., Miller, T.W., Brodeur, R.D. and Jacobson, K.C. (2008) Expanding the foraging history of juvenile Pacific salmon : combining stomach-content and macroparasite- community analyses for studying marine diets. *Journal of Fish Biology* **72**, 1268–1294.

Baluyut, E.A. (1989) Aquaculture systems and practices: a selected review. Food and Agriculture Organization of the United Nations. ADCP/REP/89/43.

Barnes, D.K. a, Galgani, F., Thompson, R.C. and Barlaz, M. (2009) Accumulation and fragmentation of plastic debris in global environments. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* **364**, 1985–1998.

Barnes, D.K.A. (2005) Remote Islands Reveal Rapid Rise of Southern Hemisphere Sea Debris. *The Scientific World Journal* **5**, 915–921.

Bartoli, M., Nizzoli, D., Viaroli, P., Turolla, E., Castaldelli, G., Fano, E.A. and Rossi, R. (2001) Impact of Tapes philippinarum farming on nutrient dynamics and benthic respiration in the Sacca di Goro. *Hydrobiologia* **455**, 203–212.

Beadman, H.A., Kaiser, M.J., Galanidi, M., Shucksmith, R. and Willows, R.I. (2004) Changes in species richness with stocking density of marine bivalves. *Journal of Applied Ecology* **41**, 464–475.

Beamish, R.. and Mahnken, C. (2001) A critical size and period hypothesis to explain natural regulation of salmon abundance and the linkage to climate and climate change. *Progress in Oceanography* **49**, 423–437.

Beamish, R.J., Mahnken, C. and Neville, C.M. (2004) Evidence That Reduced Early Marine Growth is Associated with Lower Marine Survival of Coho Salmon. *Transactions of the American Fisheries Society* **133**, 26–33.

Beamish, R.J., Sweeting, R.M., Lange, K.L. and Neville, C.M. (2008) Changes in the Population Ecology of Hatchery and Wild Coho Salmon in the Strait of Georgia. *Transactions of the American Fisheries Society* **137**, 503–520.

Beamish, R.J., Sweeting, R.M., Lange, K.L., Noakes, D.J., Preikshot, D. and Neville, C.M. (2010) Early marine survival of coho salmon in the Strait of Georgia declines to very low levels. *Marine and Coastal Fisheries* **2**, 424–439.

Beauchamp, D. (2009) Bioenergetic ontogeny: linking climate and mass-specific feeding to life-cycle growth and survival of salmon. *American Fisheries Society Symposium* **70**, 1–19.

Beck, M.W., Heck, K.L., Able, K.W., et al. (2003) The role of nearshore ecosystems as fish and shellfish nurseries. *Issues in Ecology* **11**, 1–12.

Bendell-Young, L.I. (2006) Contrasting the community structure and select geochemical characteristics of three intertidal regions in relation to shellfish farming. *Environmental Conservation* **33**, 21–27.

Biro, P.A., Post, J.R. and Parkinson, E.A. (2003) From individuals to populations: Prey fish risk-taking mediates mortality in whole-system experiments. *Ecology* **84**, 2419–2431.

Bottom, D.L., Simenstad, C.A., Burke, J., et al. (2005) Salmon at river's end: the role of the estuary in the decline and recovery of Columbia River salmon. *NOAA Technical Memorandum NMFS-NWFSC-68*, 246 pp.

Bourdon, R. (2015) Interactions between fish communities and shellfish aquaculture in Baynes Sound, British Columbia. University of Victoria.

Brodeur, R.D. (1992) Factors Related to Variability in Feeding Intensity of Juvenile Coho Salmon and Chinook Salmon. *Transactions of the American Fisheries Society* **121**, 104–114.

Brodeur, R.D. (1989) Neustonic feeding by juvenile salmonids in coastal waters of the Northeast Pacific. *Canadian Journal of Zoology* **67**, 1995–2007.

Brodeur, R.D., Daly, E.A., Sturdevant, M. V, et al. (2007) Regional Comparisons of Juvenile Salmon Feeding in Coastal Marine Waters off the West Coast of North America. *American Fisheries Society Symposium* **57**, 183–203.

Brodeur, R.D. and Pearcy, W.G. (1990) Trophic relations of juvenile Pacific salmon off the Oregon and Washington coast. *Fishery Bulletin* **88**, 617–636.

Browne, M. a., Galloway, T.S. and Thompson, R.C. (2010) Spatial patterns of plastic debris along estuarine shorelines. *Environmental Science and Technology* **44**, 3404–3409.

Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T. and Thompson, R. (2011) Accumulation of Microplastic on Shorelines Woldwide: Sources and Sinks. *Environmental Science & Technology* **45**, 9175–9179.

Bulleri, F. (2005) The introduction of artificial structures on marine soft- and hard-bottoms: ecological implications of epibiota. *Environmental Conservation* **32**, 101.

Bulleri, F. and Chapman, M.G. (2010) The introduction of coastal infrastructure as a driver of change in marine environments. *Journal of Applied Ecology* **47**, 26–35.

Carr, S.A., Liu, J. and Tesoro, A.G. (2016) Transport and fate of microplastic particles in wastewater treatment plants. *Water Research* **91**, 174–182.

Carswell, B., Cheesman, S. and Anderson, J. (2006) The use of spatial analysis for environmental assessment ofshellfish aquaculture in Baynes Sound, Vancouver Island, British Columbia, Canada. *Aquaculture* **253**, 408–414.

Chamberlain, J., Fernandes, T.F., Read, P., Nickell, T.D. and Davies, I.M. (2001) Impacts of biodeposits from suspended mussel (Mytilus edulis L.) culture on the surrounding surficial sediments. *ICES Journal of Marine Science: Journal du Conseil* **58**, 411–416.

Choy, C. and Drazen, J. (2013) Plastic for dinner? Observations of frequent debris ingestion by pelagic predatory fishes from the central North Pacific. *Marine Ecology Progress Series* **485**, 155–163.

Christensen, P.B., Glud, R.N., Dalsgaard, T. and Gillespie, P. (2003) Impacts of longline mussel farming on oxygen and nitrogen dynamics and biological communities of coastal sediments. *Aquaculture* **218**, 567–588.

Claessens, M., Meester, S. De, Landuyt, L. Van, Clerck, K. De and Janssen, C.R. (2011) Occurrence and distribution of microplastics in marine sediments along the Belgian coast. *Marine Pollution Bulletin* **62**, 2199–2204.

Clarke, K.R. (1993) Non-parametric multivariate analyses of changes in community structure. *Australian Journal of Ecology* **18**, 117–143.

Clarke, K.R. and Gorley, R.N. (2015) PRIMER v7: User Manual/Tutorial. PRIMER-E, Plymouth, 296pp.

Cluzard, M., Kazmiruk, T.N., Kazmiruk, V.D. and Bendell, L.I. (2015) Intertidal Concentrations of Microplastics and Their Influence on Ammonium Cycling as Related to the Shellfish Industry. *Archives of Environmental Contamination and Toxicology* **69**, 310–319.

Cole, M., Lindeque, P., Halsband, C. and Galloway, T.S. (2011) Microplastics as contaminants in the marine environment: A review. *Marine Pollution Bulletin* **62**, 2588–2597.

Cole, M., Lindeque, P.K., Fileman, E., Clark, J., Lewis, C., Halsband, C. and Galloway, T.S. (2016) Microplastics Alter the Properties and Sinking Rates of Zooplankton Faecal Pellets. *Environmental Science & Technology* **50**, 3239–3246.

Costa-Pierce, B.A. (2002) Ecology as the paradigm for the future of aquaculture. In: *Ecological Aquaculture: The evolution of the blue revolution*. Blackwell Science, Oxford, UK, pp 339–372.

da Costa, K.G. and Nalesso, R.C. (2006) Effects of mussel farming on macrobenthic community structure in Southeastern Brazil. *Aquaculture* **258**, 655–663.

Crain, C.M., Halpern, B.S., Beck, M.W. and Kappel, C. V. (2009) Understanding and managing human threats to the coastal marine environment. *Annals of the New York Academy of Sciences* **1162**, 39–62.

Crawford, C.M., Macleod, C.K. a and Mitchell, I.M. (2003) Effects of shellfish farming on the benthic environment. *Aquaculture* **224**, 117–140.

Daly, E.A., Brodeur, R.D. and Weitkamp, L.A. (2009) Ontogenetic Shifts in Diets of Juvenile and Subadult Coho and Chinook Salmon in Coastal Marine Waters: Important for Marine Survival? *Transactions of the American Fisheries Society* **138**, 1420–1438.

Davis, W. and Murphy, A.G. (2015) Plastic in surface waters of the Inside Passage and beaches of the Salish Sea in Washington State. *Marine Pollution Bulletin* **97**, 169–177.

Dealteris, J.T., Kilpatrick, B.D. and Rheault, R.B. (2004) A comparative evaluation of the habitat value of shellfish aquaculture gear, submerged aquatic vegetation and a non-vegetated seabed. *Journal of Shellfish Research* **23**, 867–874.

Derraik, J.G.. (2002) The pollution of the marine environment by plastic debris: a review. *Marine Pollution Bulletin* **44**, 842–852.

Desforges, J.P., Galbraith, M. and Ross, P.S. (2015) Ingestion of Microplastics by Zooplankton in the Northeast Pacific Ocean. *Archives of Environmental*

Contamination and Toxicology **69**, 320–330.

Desforges, J.P.W., Galbraith, M., Dangerfield, N. and Ross, P.S. (2014) Widespread distribution of microplastics in subsurface seawater in the NE Pacific Ocean. *Marine Pollution Bulletin* **79**, 94–99.

DFO (2013) Evaluation of Proposed Ecologically and Biologically Significant Areas in Marine Waters of British Columbia. *DFO Can. Sci. Advis. Sec. Sci. Advis. Rep. 2012/075*, 21.

DFO (1999) Lower Strait of Georgia Chinook Salmon: DFO Science Stock Status Report D6-12. **12**, 7.

DFO (1991) Strait of Georgia Coho salmon resource status and management planning process: Interim report.

Doyle, M.J., Watson, W., Bowlin, N.M. and Sheavly, S.B. (2011) Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. *Marine Environmental Research* **71**, 41–52.

Duffy, E.J., Beauchamp, D. a., Sweeting, R.M., Beamish, R.J. and Brennan, J.S. (2010) Ontogenetic Diet Shifts of Juvenile Chinook Salmon in Nearshore and Offshore Habitats of Puget Sound. *Transactions of the American Fisheries Society* **139**, 803–823.

Dumbauld, B.R., Hosack, G.R. and Bosley, K.M. (2015) Association of juvenile salmon and estuarine fish with intertidal seagrass and oyster aquaculture habitats in a northeast Pacific estuary. *Transactions of the American Fisheries Society* **144**, 1091–1110.

Emmett, R., Llansó, R., Newton, J., et al. (2000) Geographic Signatures of North American West Coast Estuaries. *Estuaries* **23**, 765.

FAO (2014) The state of world fisheries and aquaculture: Opportunities and challenges. Rome.

Fendall, L.S. and Sewell, M.A. (2009) Contributing to marine pollution by washing your face: Microplastics in facial cleansers. *Marine Pollution Bulletin* **58**, 1225–1228.

Fresh, K.L. (2006) Juvenile Pacific Salmon and the Nearshore Ecosystem of Puget Sound. *Puget Sound Nearshore Partnership*, Report No. 2006–06.

GESAMP (2015) Sources, fate & effects of microplastics in the marine environment: a global assessment. *(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*, 96p.

Giacomini, H.C., Shuter, B.J. and Lester, N.P. (2013) Predator bioenergetics and the prey size spectrum: Do foraging costs determine fish production? *Journal of Theoretical Biology* **332**, 249–260.

Grant, J., Hatcher, a., Scott, D.B., Pocklington, P., Schafer, C.T. and Winters, G. V. (1995) A Multidisciplinary Approach to Evaluating Impacts of Shellfish Aquaculture on Benthic Communities. *Estuaries* **18**, 124.

Halpern, B.S., Walbridge, S., Selkoe, K.A., et al. (2008) A Global Map of Human Impact on Marine Ecosystems. *Science* **319**, 948–952.

Hamilton, S.L., Bravender, B.A., Beggs, C. and Munro, B. (2008) Distribution and abundance of juvenile salmonids and other fish species in the Courtenay River estuary and Baynes Sound, 2001. *Canadian Technical Report of Fisheries and Aquatic Sciences* **2806**, 1–213.

Hamilton, S.L., Bravender, B.A., Munro, B., Stephens, S. and Dziekan, T. (2002) Results of beach, mini purse, purse, Gee trap, and pole seine surveys at the Courtenay River estuary and Baynes Sound, Courtenay, B.C., 2001. *Canadian Data Report of Fisheries and Aquatic Sciences*, 1–141.

Hartman, G.F. (1997) *Field Identification of Coastal Juvenile Salmonids*. Harbour Pub., University of California.

Hatcher, A., Grant, J. and Schofield, B. (1994) Effects of suspended mussel culture (Mytilus spp.) on sedimentation, benthic respiration and sediment nutrient dynamics in a coastal bay. *Marine Ecology Progress Series* **115**, 219–235.

Healey, M. (1982a) Timing and Relative Intensity of Size-Selective Mortality of Juvenile Chum Salmon (Oncorhynchus keta) During Early Sea Life. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 952–957.

Healey, M.C. (1979) Detritus and Juvenile Salmon Production in the Nanaimo Estuary: I. Production and Feeding Rates of Juvenile Chum Salmon (Oncorhynchus keta). *Journal of the Fisheries Research Board of Canada* **36**, 488–496.

Healey, M.C. (1982b) Juvenile Pacific salmon in estuaries: the life support system. In: *Estuarine Comparisons*. (ed V.S. Kennedy). Academic Press, New York, pp 315–341.

Healey, M.C. (1980) Utilization of the Nanaimo River Estuary by Juvenile Chinook Salmon Oncorhynchu Tshawytscha. *Fishery Bulletin* **77**, 653–668.

Heck, K.L.J., Hays, G. and Orth, R.J. (2003) Critical evaluation of nursery hypothesis for seagrasses. *Marine Ecology Progress Series* **253**, 123–136.

Heerhartz, S.M., Dethier, M.N., Toft, J.D., Cordell, J.R. and Ogston, A.S. (2014) Effects of Shoreline Armoring on Beach Wrack Subsidies to the Nearshore Ecotone in an Estuarine Fjord. *Estuaries and Coasts* **37**, 1256–1268.

Heerhartz, S.M. and Toft, J.D. (2015) Movement patterns and feeding behavior of juvenile salmon (Oncorhynchus spp.) along armored and unarmored estuarine shorelines. *Environmental Biology of Fishes* **98**, 1501–1511.

Heerhartz, S.M., Toft, J.D., Cordell, J.R., Dethier, M.N. and Ogston, A.S. (2015) Shoreline Armoring in an Estuary Constrains Wrack-Associated Invertebrate Communities. *Estuaries and Coasts*.

Hinojosa, I. a. and Thiel, M. (2009) Floating marine debris in fjords, gulfs and channels of southern Chile. *Marine Pollution Bulletin* **58**, 341–350.

Hosack, G.R., Dumbauld, B.R., Ruesink, J.L. and Armstrong, D. a (2006) Habitat associations of estuarine species: Comparisons of intertidal mudflat, seagrass (Zostera marina), and oyster (Crassostrea gigas) habitats. *Estuaries and Coasts* **29**, 1150–1160.

Ivar do Sul, J.A. and Costa, M.F. (2014) The present and future of microplastic pollution in the marine environment. *Environmental pollution* **185**, 352–364.

Jackson, J.B., Kirby, M.X., Berger, W.H., et al. (2001) Historical Overfishing and the Recent Collapse of Coastal Ecosystems. *Science* **293**, 629–637.

Jambeck, J.R., Geyer, R., Wilcox, C., et al. (2015) Plastic waste inputs form land into the ocean. *Science* **347**, 768–771.

Jamieson, G., Chew, L., Gillespie, G., et al. (2001) Phase 0 Review of the Environmental Impacts of Intertidal Shellfish Aquaculture in Baynes Sound. *Canadian Science Advisory Secretariat* **2001/125**, 1–103.

Jamieson, G.S. and Levesque, C. (2014) Identification of Ecologically and Biologically Significant Areas on the West Coast of Vancouver Island and the Strait of Georgia, and in some nearshore areas on the North Coast: Phase II – Designation of EBSAs.

Juanes, F., Buckel, J.A. and Scharf, F.S. (2002) Feeding ecology of piscivorous fishes. In: *Handbook of Fish Biology and Fisheries, volume 1: fish biology*. (eds P.J.B. Hart and J.D. Reynolds). Blackwell Scientific Publications, Oxford, UK, pp 267–283.

Kelly, J.R., Proctor, H. and Volpe, J.P. (2008) Intertidal community structure differs significantly between substrates dominated by native eelgrass (Zostera marina L.) and adjacent to the introduced oyster Crassostrea gigas (Thunberg) in British Columbia, Canada. *Hydrobiologia* **596**, 57–66.

Kennish, M.J. (2002) Environmental threats and environmental future of estuaries. *Environmental Conservation* **29**, 78–107.

Labelle, M., Walters, C.J. and Riddell, B. (1997) Ocean survival and exploitation of coho salmon (Oncorhynchus kisutch) stocks from the east coast of Vancouver Island, British Columbia. *Canadian Journal of Fisheries and Aquatic Sciences* **54**, 1433–1449.

Laist, D.W. (1987) Overview of the biological effects of lost and discarded plastic debris in the marine environment. *Marine Pollution Bulletin* **18**, 319–326.

Lattin, G.L., Moore, C.J., Zellers, A.F., Moore, S.L. and Weisberg, S.B. (2004) A comparison of neustonic plastic and zooplankton at different depths near the southern California shore. *Marine Pollution Bulletin* **49**, 291–294.

Levings, C.D., McAllister, C.D. and Chang, B.D. (1986) Differential use of the Campbell River estuary, British Columbia by wild and hatchery-reared juvenile Chinook salmon (Oncorhynchus tshawytscha). *Canadian Journal of Fisheries and Aquatic Sciences* **43**, 1386–1397.

Levy, D. a. and Northcote, T.G. (1982) Juvenile Salmon Residency in a Marsh Area of the Fraser River Estuary. *Canadian Journal of Fisheries and Aquatic Sciences* **39**, 270–276.

Lonnstedt, O.M. and Eklov, P. (2016) Environmentally relevant concentrations of microplastic particles influence larval fish ecology. *Science* **352**, 1213–1216.

Lotze, H.K., Lenihan, H.S., Bourque, B.J., et al. (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. *Science* **312**, 1806–1809.

Lusher, A. (2015) Microplastics in the marine environment: distribution, interactions and effects. In: *Marine Anthropogenic Litter*. (eds M. Bergmann, L. Gutow and M. Klages). Springer International Publishing, pp 245–307.

Magnusson, A. and Hilborn, R. (2003) Estuarine influence on survival rates of coho (Oncorhynchus kisutch) and chinook salmon (Oncorhynchus tshawytscha) released from hatcheries on the U.S. Pacific coast. *Estuaries* **26**, 1094–1103.

Mathalon, A. and Hill, P. (2014) Microplastic fibers in the intertidal ecosystem surrounding Halifax Harbor, Nova Scotia. *Marine Pollution Bulletin* **81**, 69–79.

Mato, Y., Isobe, T., Takada, H., Kanehiro, H., Ohtake, C. and Kaminuma, T. (2001) Plastic resin pellets as a transport medium for toxic chemicals in the marine environment. *Environmental Science and Technology* **35**, 318–324.

Mattila, J., Heck, K.L., Millstein, E., Miller, E., Gustafsson, C., Williams, S. and Byron, D. (2008) Increased habitat structure does not always provide increased refuge from predation. *Marine Ecology Progress Series* **361**, 15–20.

Mazurais, D., Ernande, B., Quazuguel, P., et al. (2015) Evaluation of the impact of polyethylene microbeads ingestion in European sea bass (Dicentrarchus labrax) larvae. *Marine Environmental Research* **112**, 1–8.
McCormick, M.I. (1994) Comparison of field methods for measuring surface topography and their associations with a tropical reef fish assemblage. *Marine Ecology Progress Series* **112**, 87–96.
McKindsey, C.W., Archambault, P., Callier, M.D. and Olivier, F. (2011) Influence of suspended and off-bottom mussel culture on the sea bottom and benthic habitats: a review. *Canadian Journal of Zoology* **89**, 622–646.
Meehan, W.R. and Miller, R.A. (1978) Stomach Flushing: Effectiveness and Influence on Survival and Condition of Juvenile Salmonids. *Journal of the Fisheries Research Board of Canada* **35**, 1359–1363.
Miller, B. a. and Sadro, S. (2003) Residence Time and Seasonal Movements of Juvenile Coho Salmon in the Ecotone and Lower Estuary of Winchester Creek, South Slough, Oregon. *Transactions of the American Fisheries Society* **132**, 546–559.
Moore, C.J., Moore, S.L., Weisberg, S.B., Lattin, G.L. and Zellers, A.F. (2002) A comparison of neustonic plastic and zooplankton abundance in southern California's coastal waters. *Marine Pollution Bulletin* **44**, 1035–1038.
Moreira, F.T., Prantoni, A.L., Martini, B., de Abreu, M.A., Stoiev, S.B. and Turra, A. (2015) Small-scale temporal and spatial variability in the abundance of plastic pellets on sandy beaches: Methodological considerations for estimating the input of microplastics. *Marine Pollution Bulletin* **102**, 114–121.
Morley, S.A., Toft, J.D. and Hanson, K.M. (2012) Ecological Effects of Shoreline Armoring on Intertidal Habitats of a Puget Sound Urban Estuary. *Estuaries and Coasts* **35**, 774–784.
Munroe, D. and McKinley, R.S. (2007) Commercial Manila clam (Tapes philippinarum) culture in British Columbia, Canada: The effects of predator netting on intertidal sediment characteristics. *Estuarine, Coastal and Shelf Science* **72**, 319–328.
Munsch, S.H., Cordell, J.R. and Toft, J.D. (2015) Effects of seawall armoring on juvenile Pacific salmon diets in an urban estuarine embayment. *Marine Ecology Progress Series* **535**, 213–229.
Newell, R. (2004) Ecosystem influence of natural cultivated populations of suspension-feeding bivalve molluscs: a review. *Journal of Shellfish Research* **23**, 51–61.
Newell, R. and Koch, E.W. (2004) Modeling Seagrass Density and Distribution in Response to Changes in Turbidity Stemming from Bivalve Filtration and Seagrass Sediment Stabilization. *Estuaries* **27**, 793–806.
Nguyen, T. and Williams, T. (2013) Aquaculture in Canada. Ottawa, Canada.
Parker, R.R. (1968) Marine Mortality Schedules of Pink Salrnon of the Bella Coola River. Central British Colurnbia. *Journal Fisheries Research Board of Canada* **5**, 757–794.
Pearcy, W.G. (1992) Ocean ecology of North Pacific salmonids. *Environmental Biology of Fishes* **42**, 105–108.
Pedà, C., Caccamo, L., Fossi, M.C., et al. (2016) Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: Preliminary results. *Environmental Pollution* **212**, 251–256.
Phillips, R.C. (1984) Ecology of eelgrass meadows in the Pacific northwest: A

community profile. *U.S. Fish and Wildlife Service* **FWS/OBS-84**, 85 pp.
PlasticsEurope (2015) Plastics - the facts 2015. Brussels, Belgium.
Powers, M., Peterson, C., Summerson, H. and Powers, S. (2007) Macroalgal growth on bivalve aquaculture netting enhances nursery habitat for mobile invertebrates and juvenile fishes. *Marine Ecology Progress Series* **339**, 109–122.
Quayle, D.B. (1964) Distribution of introduced marine mollusca in British Columbia waters. *Journal of the Fisheries Research Board of Canada* **21**, 1155–1181.
Quinn, T.P. (2005) *The behavior and ecology of Pacific salmon and trout*. UBC Press.
R Core Team (2014) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/.
Rochman, C.M., Cook, A. and Koelmans, A.A. (2016) Plastic debris and policy: Using current scientific understanding to invoke positive change. *Environmental Toxicology and Chemistry* **35**, 1617–1626.
Rochman, C.M., Hoh, E., Kurobe, T. and Teh, S.J. (2013) Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. *Scientific reports* **3**, 3263.
Rochman, C.M., Tahir, A., Williams, S.L., et al. (2015) Anthropogenic debris in seafood: Plastic debris and fibers from textiles in fish and bivalves sold for human consumption. *Scientific Reports* **5**, 1–10.
Romanuk, T.N. and Levings, C.D. (2005) Stable isotope analysis of trophic position and terrestrial vs. marine carbon sources for juvenile Pacific salmonids in nearshore marine habitats. *Fisheries Management and Ecology* **12**, 113–121.
Ruesink, J.L., Lenihan, H.S., Trimble, A.C., Heiman, K.W., Micheli, F., Byers, J.E. and Kay, M.C. (2005) Introduction of non-native oysters: Ecosystem Effects and Restoration Implications. *Annual Review of Ecology, Evolution, and Systematics* **36**, 643–689.
Schabetsberger, R., Morgan, C. a., Brodeur, R.D., Potts, C.L., Peterson, W.T. and Emmett, R.L. (2003) Prey selectivity and diel feeding chronology of juvenile chinook (Oncorhynchus tshawytscha) and coho (O. kisutch) salmon in the Columbia River plume. *Fisheries Oceanography* **12**, 523–540.
Setälä, O., Fleming-Lehtinen, V. and Lehtiniemi, M. (2014) Ingestion and transfer of microplastics in the planktonic food web. *Environmental Pollution* **185**, 77–83.
Simenstad, C. and Fresh, K. (1995) Influence of intertidal aquaculture on benthic communities in pacific northwest estuaries: scales of disturbance. *Estuaries* **18**, 43.
Simenstad, C.A. and Cordell, J.R. (2000) Ecological assessment criteria for restoring anadromous salmonid habitat in Pacific Northwest estuaries. *Ecological Engineering* **15**, 283–302.
Simenstad, C.A., Cordell, J.R. and Weitcamp, L.A. (1993) Effects of substrate modification on littoral flat epibenthos: assemblage structure changes associated with predator exclusion nets. *Technical report to Washington Department of Fisheries, Point Whitney Shellfish Laboratory, Brinnon, Washington* **FRI-UW-931**.
Simenstad, C.A., Fresh, K.L. and Salo, E.O. (1982) the Role of Puget Sound and Washington Coastal Estuaries in the Life History of Pacific Salmon: an Unappreciated Function. *Estuarine Comparisons*, 343–364.
Sogard, S.S.M. (1997) Size-selective mortality in the juvenile stage of teleost fishes: a review. *Bulletin of Marine Science* **60**, 1129–1157.

Solomon, O.O. and Palanisami, T. (2016) Microplastics in the Marine Environment: Current Status, Assessment Methodologies, Impacts and Solutions. *Journal of Pollution Effects & Control* **04**, 1–13.
Sorokin, I.I., Giovanardi, O., Pranovi, F. and Sorokin, P.I. (1999) Need for restricting bivalve culture in the southern basin of the Lagoon of Venice. *Hydrobiologia* **400**, 141–148.
Spencer, B.E., Kaiser, M.J. and Edwards, D.B. (1997) Ecological effects of intertidal manila clam cultivation at the end of the cultivation phase. *Journal of Applied Ecology* **34**, 444–452.
Strand, J., Lassen, P., Shashoua, Y. and Andersen, J.H. (2013) Microplastic particles in sediments from Danish waters In: Poster at the ICES Annual conference Reykjavik, Iceland.
Teuten, E.L., Saquing, J.M., Knappe, D.R.U., et al. (2009) Transport and release of chemicals from plastics to the environment and to wildlife. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**, 2027–2045.
Thompson, R.C., Olsen, Y., Mitchell, R.P., et al. (2004) Lost at sea: where is all the plastic? *Science (New York, N.Y.)* **304**, 838.
Thorpe, J.E. (1994) Salmonid fishes and the estuarine environment. *Estuaries And Coasts* **17**, 76–93.
Toft, J.D., Cordell, J.R., Simenstad, C.A. and Lia A. Stamatiou (2007) Fish Distribution, Abundance, and Behavior along City Shoreline Types in Puget Sound. *North American Journal of Fisheries Management* **27**, 465–480.
Toft, J.D., Ogston, A.S., Heerhartz, S.M., Cordell, J.R. and Flemer, E.E. (2013) Ecological response and physical stability of habitat enhancements along an urban armored shoreline. *Ecological Engineering* **57**, 97–108.
Truscott, J. (2002) The Baynes Sound coastal plan for shellfish aquaculture.
VanCauwenberghe, L., Devriese, L., Galgani, F., Robbens, J. and Janssen, C.R. (2015) Microplastics in sediments: A review of techniques, occurrence and effects. *Marine Environmental Research* **111**, 5–17.
VanCauwenberghe, L. and Janssen, C.R. (2014) Microplastics in bivalves cultured for human consumption. *Environmental pollution* **193**, 65–70.
VanCauwenberghe, L., Vanreusel, A., Mees, J. and Janssen, C.R. (2013) Microplastic pollution in deep-sea sediments. *Environmental Pollution* **182**, 495–499.
Vethaak, A.D. and Leslie, H.A. (2016) Plastic Debris Is a Human Health Issue. *Environmental Science & Technology* **50**, 6825–6826.
Vianello, A., Boldrin, A., Guerriero, P., Moschino, V., Rella, R., Sturaro, A. and Da Ros, L. (2013) Microplastic particles in sediments of Lagoon of Venice, Italy: First observations on occurrence, spatial patterns and identification. *Estuarine, Coastal and Shelf Science* **130**, 54–61.
Willson, M. and Hulupka, K. (1995) Anadromous fishes as keystone species in vertebrate communities. *Conservation Biology* **9**, 489–497.
Wright, S.L., Thompson, R.C. and Galloway, T.S. (2013) The physical impacts of microplastics on marine organisms: A review. *Environmental Pollution* **178**, 483–492.
Zarfl, C. and Matthies, M. (2010) Are marine plastic particles transport vectors for organic pollutants to the Arctic? *Marine Pollution Bulletin* **60**, 1810–1814.

Appendix

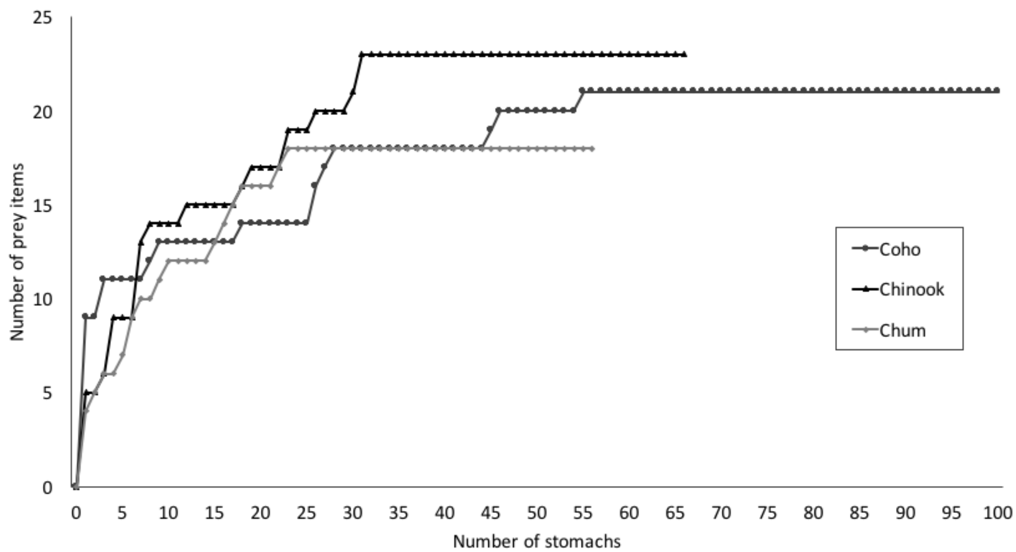

Figure A9: Cumulative prey curves plotted for Coho, Chinook and Chum salmon. The cumulative number of prey items identified is on the y-axis while the cumulative number of stomach samples analyzed is on the x-axis.

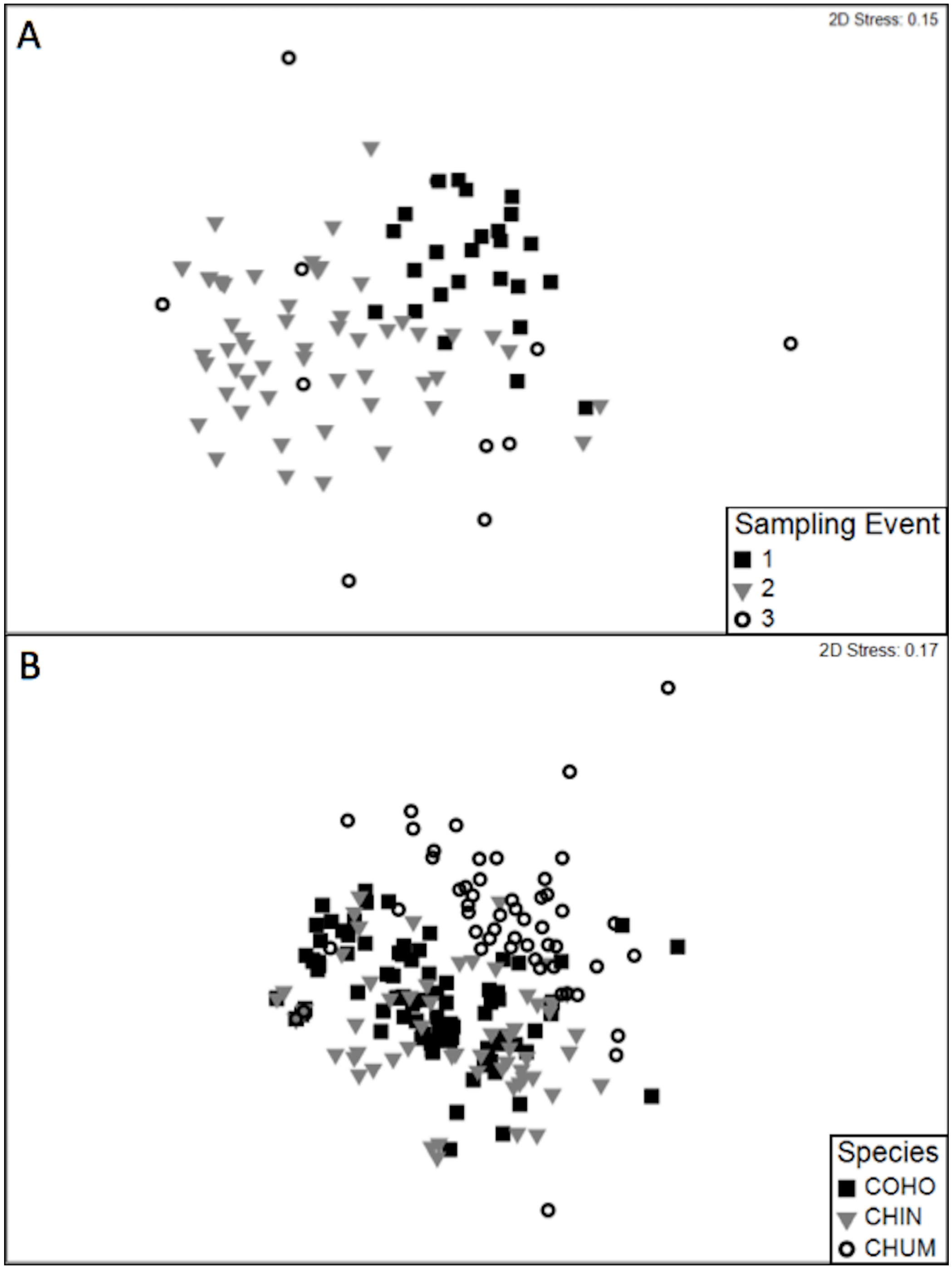

Figure A10: Non-metric multidimensional scaling ordination of Coho prey abundance by sampling event (A) and all species prey abundance (B). Salmon were captured in intertidal areas across three aquaculture sites and three corresponding non-aquaculture sites from May – July, 2014 using a beach seine.