

Eelgrass habitat as near-shore foraging grounds for juvenile Pacific salmon

by

Laura Kennedy  
BSc, University of Northern British Columbia, 2011

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

MASTER OF SCIENCE

in the Department of Biology

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## **Supervisory Committee**

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## **Supervisory Committee**

Dr. Rana El-Sabaawi, Department of Biology  
**Supervisor**

Dr. Francis Juanes, Department of Biology  
**Supervisor**

Dr. Julia Baum, Department of Biology  
**Departmental Member**

## Abstract

The early marine period for juvenile salmon is a critical life history stage for growth and survival. The health of near-shore ecosystems where juvenile salmon spend their early marine life, and the capacity of these habitats to provide prey are thus important for overall salmon returns. While near-shore habitat is generally accepted as being of critical importance, few studies have examined how juvenile salmon use this habitat during their early marine life. Understanding the trophic dynamics in these ecosystems and the characteristics of these habitats that are important for food provision will help us gauge the value of near-shore areas to the early marine phase of juvenile salmon. The importance of this avenue of research is underscored by declined rates of early marine survival of salmon, and suggestions that the rapid development of the shoreline is contributing to this decreased survival.

My project investigates the role that near-shore habitat plays in promoting growth and survival of juvenile salmon by evaluating eelgrass as foraging grounds for juvenile salmon in the Comox Estuary, British Columbia. In 2015, we compared juvenile Chum salmon diets to prey availability in zooplankton tows and in epifaunal and infaunal eelgrass samples across a gradient of eelgrass shoot density. We complemented diet analysis with the use of stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) to examine the relative contribution of zooplankton, eelgrass, and terrestrial invertebrates to juvenile Chum salmon diet. In 2016, we collected additional eelgrass samples to cover a wider range of eelgrass density, and compared juvenile Chinook salmon diets to prey availability in eelgrass.

Prey invertebrates, such as polychaete worms, harpacticoid copepods, and amphipods, increased with eelgrass shoot density. Juvenile Chum salmon and juvenile Chinook salmon assessed in this study fed primarily on benthic species, specifically on harpacticoid copepods, which were only abundant in epifaunal eelgrass samples. The abundance of prey invertebrates in eelgrass beds and the benthic diet of juvenile salmon in the Comox Estuary leads us to conclude that the presence of eelgrass is an important habitat feature for juvenile salmon.

## Table of Contents

Supervisory Committee.....	i
Abstract.....	ii
List of Tables.....	iv
List of Supplementary Tables.....	vi
List of Figures.....	vii
List of Supplementary Figures.....	ix
Acknowledgements.....	x
<b>Chapter 1: Project Overview.....</b>	<b>1</b>
<b>THESIS INTRODUCTION.....</b>	<b>1</b>
<b>THE SALISH SEA MARINE SURVIVAL PROJECT.....</b>	<b>2</b>
<b>EELGRASS ECOLOGY.....</b>	<b>4</b>
<b>STUDY SPECIES.....</b>	<b>8</b>
<b>JUVENILE SALMON IN ESTUARIES.....</b>	<b>12</b>
<b>STABLE ISOTOPE ANALYSIS.....</b>	<b>14</b>
<b>SUMMARY.....</b>	<b>17</b>
<b>LITERATURE CITED.....</b>	<b>18</b>
<b>Chapter 2: Eelgrass as Foraging Habitat for Juvenile Salmon.....</b>	<b>26</b>
<b>GENERAL PROJECT INTRODUCTION.....</b>	<b>26</b>
<b>METHODS.....</b>	<b>31</b>
<b>RESULTS.....</b>	<b>51</b>
<b>DISCUSSION.....</b>	<b>77</b>
<b>SUMMARY.....</b>	<b>87</b>
<b>LITERATURE CITED.....</b>	<b>90</b>
<b>Chapter 3: Management Relevance.....</b>	<b>100</b>
<b>SALMON POPULATIONS IN THE SALISH SEA.....</b>	<b>100</b>
<b>EELGRASS HABITAT IN THE SALISH SEA.....</b>	<b>103</b>
<b>MANAGEMENT IMPLICATIONS AND FUTURE RESEARCH.....</b>	<b>105</b>
<b>LITERATURE CITED.....</b>	<b>109</b>
<b>Appendix.....</b>	<b>112</b>
<b>SUPPLEMENTARY TABLES.....</b>	<b>112</b>
<b>SUPPLEMENTARY FIGURES.....</b>	<b>116</b>

## List of Tables

- Table 1. Eelgrass variables in low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015-2016.
- Table 2. Average and standard deviation (sd) of eelgrass prey invertebrate counts in low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015-2016. Averages are rounded to the nearest whole number.
- Table 3. Pearson correlations between variables of eelgrass collected from the Comox Estuary, British Columbia, May 2015-2016. Significant correlations are in bold.
- Table 4. Summary of negative binomial model of epifaunal prey invertebrate abundance in eelgrass quadrats from the Comox Estuary, British Columbia, May 2015-2016.
- Table 5. Average and standard deviation (sd) of shoots/m<sup>2</sup> and average, standard deviation, and frequency of occurrence (FO) of infaunal prey invertebrate counts in 10cm deep, 8cm diameter sediment cores from quadrats in low (n=11), medium (n=5), and high (n=13) eelgrass shoot density categories in the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.
- Table 6. Summary of negative binomial model of infaunal prey invertebrate abundance as a function of shoot density, sediment depth, and percent fine sediment in the Comox Estuary, British Columbia, May 2015.
- Table 7. Temperature, salinity, and bottom depth of zooplankton tows, and average, standard deviation (sd) and total density/m<sup>3</sup> (Total) of zooplankton caught in inner-estuary tows over eelgrass and unvegetated sediment, and tows outside the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.
- Table 8. Species occurrences in daytime (15:00-18:00) and night-time (21:00-23:00) invertebrate sweeps over eelgrass in the Comox Estuary, British Columbia, May 2016.
- Table 9. Salmon metrics, average, standard deviation (sd), and frequency of occurrence (FO) of invertebrate counts in juvenile Chum salmon diets by tow number from the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.
- Table 10. Average, standard deviation (sd), and frequency of occurrence (FO) of invertebrate counts in Chinook salmon diets by tow number from the Comox

Estuary, British Columbia, May 2016. Averages are rounded to the nearest whole number.

Table 11. Average, standard deviation (sd), and sample size (n) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope signatures and trophic enrichment factors for juvenile Chum salmon and potential diet sources and resources in the Comox Estuary, British Columbia, May 2015.  $\delta^{13}\text{C}$  signatures of juvenile Chum salmon have been lipid corrected.

## List of Supplementary Tables

- Table S1. Average and standard deviation (sd) of eelgrass invertebrate counts in low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015-2016. Averages are rounded to the nearest whole number.
- Table S2. Summary of negative binomial model of prey invertebrate abundance, with apparent outlier removed, in eelgrass quadrats from the Comox Estuary, British Columbia, May 2015-2016.
- Table S3. Results of model selection of eelgrass variables to predict epifaunal invertebrate abundance per 0.0625m<sup>2</sup> quadrat using a negative binomial model. All variables have been standardized to unit variance with a mean of 0.
- Table S4. Average and standard deviation (sd) of shoots/m<sup>2</sup> and average, standard deviation, and frequency of occurrence (FO) of infaunal invertebrate counts in 10cm deep, 8cm diameter sediment cores from quadrats in low (n=11), medium (n=5), and high (n=13) eelgrass shoot density categories in the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.
- Table S5. Pearson correlations between  $\delta^{13}\text{C}$  signatures, fork length, weight, and C:N ratios of juvenile Chum salmon caught in the Comox Estuary, British Columbia, May 2015.
- Table S6. Mean, standard deviation, and Bayesian credible intervals for estimated diet proportions of food sources to the diets of juvenile Chum salmon in the Comox Estuary using uninformative and informative priors.

## List of Figures

- Figure 1. Map of 2015/2016 sampling in the Comox Estuary, British Columbia, Canada.
- Figure 2. Predicted values of negative binomial model of epifaunal prey invertebrate abundance as a function of shoot density in 0.0625m<sup>2</sup> eelgrass quadrats collected in May 2015 and 2016 in the Comox Estuary, British Columbia. Year is held at its centered value, and epiphyte biomass at its mean.
- Figure 3. Constrained analysis of principal coordinates (CAP) of epifaunal prey invertebrate communities across low, medium, and high-density eelgrass collected from 0.0625m<sup>2</sup> quadrats in May 2015-2016 from the Comox Estuary, British Columbia. Shoot density, epiphyte biomass, and year are explanatory variables, with length of the arrows indicating the strength of each predictor. Each point on the graph represents the epifaunal prey invertebrates found in a single quadrat, with density categories coded as colours and year as different shapes. Species are abbreviated for display purposes as follows: Gam = gammarid amphipods, Senti = other senticaud amphipods, Amph = other amphipods, Tana = tanaids, Coro = corophid amphipods, Poly = polychaete worms, Iso = isopods, Mite = mites, Harp = harpacticoid copepods, Cuma = cumaceans. Shoot density is shoots/0.0625m<sup>2</sup> quadrat.
- Figure 4. Constrained analysis of principal coordinates (CAP) of infaunal prey invertebrate communities in sediment cores from low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015, with eelgrass shoot density, sediment depth, and percent of fine sediments as explanatory variables. Eelgrass density categories are coded as different colours, and each point on the graph represents the infaunal invertebrates found in a single sediment core. Species are abbreviated as follows: Gam = gammarid amphipods, Amph = other amphipods, Tana = tanaids, Poly = polychaete worms, Iso = isopods, Harp = harpacticoid copepods, Cuma = cumaceans, worm = other benthic annelids. Shoot density is shoots/0.0625m<sup>2</sup> in the quadrat sediment cores were collected from, fine sediment is the dry weight of sediment <125 µm/ total dry weight of sediment.
- Figure 5. Constrained analysis of principal coordinates (CAP) of zooplankton communities from tows over eelgrass in vegetated and unvegetated estuary, and outer-estuary in Comox British Columbia, May 2015. Tow type is the explanatory variable, and is coded as different colours, with each point on the graph representing the invertebrates found in a single tow. Species are abbreviated for display purposes as follows: Amph = other amphipods, Poly = polychaete worms, Iso = isopods, Harp = harpacticoid copepods, Ins = terrestrial insects, Naup = crustacean nauplii, Hyp = hyperid amphipods, Cyp = barnacle cyprids, Mega = crab megalopae, Snail = sea snails, C zoea = crab zoea, Shrimp

= shrimp zoea, Fish = larval fish, Cala = calanoid copepods, Clad = cladocerans, Euph = euphasid zoea, Cyclo = cyclopoid copepod, Cyph = cyphanaules larvae.

Figure 6. Species accumulation curves for diets of juvenile Chum salmon caught in May 2015, and juvenile Chinook salmon caught in May, 2016 in the Comox Estuary, British Columbia.

Figure 7. Average proportion of invertebrate counts in the diets of juvenile Chum salmon caught in 2015 and juvenile Chinook salmon caught in 2016 from the Comox Estuary, British Columbia.

Figure 8. Length of copepods found in juvenile Chum salmon stomachs, zooplankton, and epifaunal and infaunal eelgrass samples from the Comox Estuary, British Columbia, May 2015.

Figure 9.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of juvenile Chum salmon and three potential carbon sources from the Comox Estuary, British Columbia, 2015-2016. Juvenile Chum salmon have been corrected for lipids and a trophic enrichment factor of 0.4 for  $\delta^{13}\text{C}$  and 3.4 for  $\delta^{15}\text{N}$ .

## **List of Supplementary Figures**

Figure S1. Histogram of total epifaunal prey invertebrate abundance in 0.0625m<sup>2</sup> eelgrass quadrats from the Comox Estuary, BC, May 2015-2016.

Figure S2. Histogram of total infaunal prey invertebrate abundance in infaunal eelgrass samples from low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015-2016.

Figure S3. Predicted values of negative binomial model of epifaunal prey invertebrate abundance as a function of shoot density in 0.0625m<sup>2</sup> eelgrass quadrats collected in May 2015 and 2016 in the Comox Estuary, British Columbia. Year is held at its centered value, and epiphyte biomass at its mean, and a potential outlier was removed.

Figure S4. Average proportion of invertebrate weight in diets of small and large juvenile Chum salmon caught in the Comox Estuary, British Columbia, 2015.

## **Acknowledgements**

It was a privilege to have been co-supervised by Dr. Rana El-Sabaawi and Dr. Francis Juanes. I would like to thank them both for providing me with invaluable scientific insight, guidance, and encouragement. I could not have imagined a better learning environment in which to spend the last few years invested in this project. Thank you. I would also like to thank my committee member Dr. Julia Baum for bringing her statistical expertise to my project, and allowing me to be involved in her exciting eelgrass program. To the El-Sabaawi, Juanes, and Baum labs, thanks for the discussions and the cookie breaks, it's been fun to have been part of such a diverse group of young scientists. Thank you especially to Geoffrey Osgood for his amazing statistical help, without which I would not be here in one piece. I would also like to acknowledge Mary Toews, Robert Bourdon, Lily Campbell, Will Duguid, and Robert Sainsbury, for their assistance and enthusiasm in the field (especially Mary who let me drag my dirty field equipment and exhausted field assistants through her house, two years in a row). Thank you to Project Watershed and the SeaChange society for helping me guide this project towards obtaining information pertinent to eelgrass and salmon conservation, and to Dr. Moira Galbraith for help with species identification. The Pacific Salmon Foundation funded this project, and I would like to thank Dr. Brian Riddell and Dr. Isobel Pearsall for their continued support. Finally, to my friends and family, who make it all worth it in the end.

# Chapter 1: Project Overview

## THESIS INTRODUCTION

Growth and survival during the early marine stage of juvenile salmon is a powerful determinant of successful spawning populations (Beamish and Mahnken 2001). The early marine life of juvenile salmon is spent in near-shore habitats (Duffy et al. 2005), meaning the capacity of these areas to support juvenile salmon growth is important for sustaining healthy salmon populations (Beamish et al. 2010). Few studies, however, have examined how juvenile salmon use near-shore areas in their early marine life, and what characteristics of these habitats support growth through food provision (Weitkamp et al. 2014). Eelgrass (*Zostera marina* L.) beds, a common habitat feature in near-shore ecosystems in the Salish Sea (Wright et al. 2014), have been proposed as important feeding grounds for juvenile salmon (Weitkamp et al. 2014). This study examines links between eelgrass habitat and juvenile salmon diets in the Comox Estuary, a sheltered bay within the Salish Sea. We hypothesized that eelgrass habitat would host an abundance of prey items targeted by juvenile salmon, and that prey availability would increase with increasing eelgrass density. Understanding the trophic dynamics in these ecosystems is becoming increasingly important given recent declines in juvenile salmon early marine survival (Beamish et al. 2003), and impending losses of eelgrass habitat in the Salish Sea (Short et al. 2010).

In Chapter 1 we provide background information for the basis of this research project. It begins with an overview of the Salish Sea Marine Survival Project, of which this study is

a part, and summarizes eelgrass and salmon biology and ecology in the Salish Sea. In Chapter 2 we present our study on eelgrass as foraging habitat for juvenile salmon. In 2015, we compared juvenile Chum salmon diets to prey availability in zooplankton, and in eelgrass blades (epifaunal eelgrass samples) and sediments (infaunal eelgrass samples) across a gradient of eelgrass density. We complemented diet analyses with the use of stable isotope ratios of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) to examine the relative contribution of zooplankton, epifaunal eelgrass invertebrates, and terrestrial invertebrates to juvenile Chum salmon diets. In 2016, we collected additional epifaunal eelgrass samples to assess invertebrates across a wider range of eelgrass shoot density, and caught juvenile Chinook salmon to investigate the presence of eelgrass-associated invertebrates in their diets. In Chapter 3 we offer an introduction to the challenges of salmon and eelgrass management in the Salish Sea and discuss the possible management implications of our study, suggesting conservation opportunities and future research directions.

## **THE SALISH SEA MARINE SURVIVAL PROJECT**

The Salish Sea Marine Survival Project (SSMSP) is a collaborative research effort designed to respond to precipitous declines of Chinook salmon and Coho salmon in the Salish Sea. Managed by the Pacific Salmon Foundation in British Columbia, Canada, and Seattle-based Long Live The Kings in the United States, the SSMSP is a 10 million dollar project involving a network of academic, non-profit, First Nations, industry, and government stakeholders. One of the overarching goals of the project is to restore wild stocks of Pacific salmon and Steelhead in the Salish Sea, a network of coastal water bodies including Puget Sound, the Strait of Georgia, and the Strait of Juan de Fuca. Other

goals of the SSMSP are to maintain sustainable fisheries, increase their value, and establish effective ecosystem science-based monitoring of the Salish Sea environment and the salmon populations that utilize it. The main objective in fulfilling these goals is to determine what is affecting the marine survival of juvenile Pacific salmon and Steelhead in the Salish Sea, which have experienced drastically reduced survival rates since the 1970s (Beamish et al. 1995, Beamish et al. 2010). The SSMSP investigates how the causes of these declines are linked to top-down processes, including predators, competition, and diseases, and to bottom-up processes such as prey availability (Riddell 2015).

A major component of understanding the effect of bottom-up processes on juvenile salmon is determining the role that habitat plays in providing productive foraging grounds to support salmon growth and survival. The SSMSP acknowledges the current lack of knowledge of how juvenile salmon interact with their near-shore habitat, and how different habitats influence prey availability for juvenile salmon. Our project is funded through the SSMSP, and we address this knowledge gap by evaluating the capacity of eelgrass to supply an abundance of invertebrate prey in the Comox Estuary, British Columbia. We also aimed to determine whether juvenile salmon from the Puntledge River utilized these invertebrates. Our first objective was to understand how infaunal and epifaunal prey invertebrate communities changed in abundance and structure with variation in eelgrass density and form. To address this objective, we sampled eelgrass variables and potential salmon prey invertebrates in eelgrass blades and sediment cores across a gradient of eelgrass density. We also conducted zooplankton tows to compare

prey availability over eelgrass and unvegetated sediment in the Comox Estuary. We hypothesized that eelgrass would host an abundance of infaunal and epifaunal prey invertebrates, and that prey invertebrate counts and richness would increase with increasing eelgrass density. We also hypothesized that the presence of eelgrass would increase eelgrass-associated prey availability in the water column. The second objective was to determine the importance of eelgrass prey invertebrates in juvenile salmon diets. We used stomach content analysis of juvenile Chinook salmon, and a combination of stomach content analysis and stable isotope analysis of juvenile Chum salmon to determine the importance of eelgrass prey invertebrates in their diets. We hypothesized that both salmon species would have an abundance of eelgrass invertebrates in their stomach contents, and that juvenile Chum salmon signatures would reflect those of their eelgrass invertebrate prey. The following section offers a general introduction to eelgrass and juvenile salmon in the Salish Sea, and provides background for the development of our hypotheses, and the project design outlined in Chapter 2.

## **EELGRASS ECOLOGY**

Early studies on the biology of *Zostera marina* L. (eelgrass) provided the groundwork for understanding eelgrass ecology, and are summarized by Phillips (1984). Eelgrass is a flowering marine plant that populates the soft sediments of many estuaries worldwide, extending into North America from the Gulf of California to the Bering Sea. Eelgrass depth depends on a variety of factors, including light availability and exposure, but generally ranges between +1.6m to -6.6m. Dense root-rhizome matrices, 3-4cm deep, anchor eelgrass shoots to the sediment and allow for the growth of strap-like leaf blades

that reach up to 2m tall. Eelgrass can reproduce both vegetatively and via flowering shoots, with higher frequency of flowering in intertidal areas. These intertidal areas are more associated with increased stress due to wave action, radiation, grazing, and fluctuations in temperature and salinity, which promote an r-selective reproduction strategy. These eelgrass life history features influence near-shore ecosystem dynamics in a variety of ways, making eelgrass an important habitat feature for many coastal species (Orth et al. 2006).

It is widely recognized that seagrasses in general host higher abundances of epifaunal invertebrates than adjacent unvegetated areas (see Orth et al. 1984). Sediment-stabilizing properties of seagrass encourage larval settlement (Bostrom and Bonsdorff 2000), while seagrass leaves increase vertical structure and create habitat niches that promote diversity and density of invertebrates (Orth et al. 1984). This effect has been demonstrated in eelgrass beds across much of their range (eg. in the Baltic Sea (Moller et al. 2014), the North Atlantic (Atrill et al. 2000, Heck et al. 1995), and the Northeastern Pacific Ocean (Hosack et al. 2006)). Comparisons of invertebrate communities among areas, however, can be difficult due to the vast variability in eelgrass habitats. For example, eelgrass can exist as continuous beds or as patches with distinct boundaries (Phillips et al. 1983). Invertebrate composition and density display edge effects in seagrass habitats (Bell et al. 2001), and can vary with patch size and shape (Bologna and Heck 2002) and location in the estuary (Bowden et al. 2001). Reproduction in eelgrass (the amount of vegetative versus flowering shoots) also varies with latitude (Phillips et al. 1983), and may be affected by depth, water currents, and sediment characteristics that can cause differences

in reproductive effort even between sites in the same area (Cabaco and Santos 2010). Invertebrate abundance is higher in flowering than vegetative shoots (Carr et al. 2011). The amount of vegetative shoots present in an eelgrass bed also can vary between tidal and subtidal sites, making comparisons between the two site types difficult (Phillips et al. 1983). Algal assemblages and epiphyte load in eelgrass can also vary among sites within a few kilometers of one another (Saunders et al. 2003), which can affect invertebrate community structure (Douglass et al. 2007). Fish predation also plays an important role in controlling community structure in eelgrasses (Nelson et al. 1979). In turn, fish communities are sensitive to a multitude of abiotic factors that can differ between eelgrass beds (Robinson et al. 2011). High variability between eelgrasses in different locations underscores the need to increase local knowledge of eelgrass communities on a site-specific basis. Although eelgrass is one of the most widely studied seagrasses (Bostrom et al. 2006), there are only a limited number of studies describing the relationship between invertebrate abundances and eelgrass density in North America (Knight et al. 2015). Understanding eelgrass communities in a more local basis is an endeavor that is especially important in the face of rapid global eelgrass loss (Orth et al. 2006).

Eelgrass habitat disturbances occur across a range of scales, from localized damage by anchors, to larger scale removal via industrial activities such as fishing (Neckles et al. 2005) or construction (Tuya et al. 2002). Ecosystem-wide habitat disturbances, such as eelgrass die off due to turbidity (Moore et al. 1996), eutrophication (Orth et al. 2006), or ocean warming (Ehlers et al. 2008), can also occur. Even within these disturbance scales,

however, there can be a of range eelgrass loss. For example, the extent of anchor damage depends on the type of anchor used, the presence of an anchor chain, long-term or short-term mooring, and the size of the boat (Boese et al. 2009). Because eelgrass loss occurs on a range of scales, an objective of this project was to describe prey invertebrate communities across a gradient of eelgrass density. In order to fulfill this objective, we sampled eelgrass and attached invertebrates across a range of eelgrass density in the Comox Estuary, an important rearing area for juvenile salmon (Tryon 2011). We hypothesized that epifaunal eelgrass invertebrates would increase with eelgrass density. By demonstrating the relationship between eelgrass prey invertebrates and shoot density on a continuous scale, we offer a more clear understanding of ecosystem dynamics than possible with a simple assessment of invertebrate abundance based on the presence or absence of eelgrass. Such information may help better predict potential impacts of eelgrass loss, and how it may affect juvenile salmon, across the wide scale of disturbances facing eelgrass habitat.

As well as supporting increased above-ground invertebrate abundances, eelgrass sediments are an important habitat for infaunal species (Bostrom and Bonsdorff 1997, Orth et al. 1984). The ability of eelgrass to stabilize sediment allows for faster accumulation of organic matter, which nourishes and promotes the growth of infauna (Bostrom and Bonsdorff 1997, Kelly et al. 2008). The complex root-rhizome matrix of eelgrass also creates habitat niches that serve to increase infaunal abundance (Orth et al. 1984). Because infaunal invertebrates may be equally as important to juvenile salmon prey availability as epifaunal prey invertebrates, we also collected sediment core samples

across a range of eelgrass density. These infaunal eelgrass samples allowed us to test our hypothesis that eelgrass density increased prey invertebrate density in the sediment.

Individuals that benefit from seagrass habitats are not necessarily spatially exclusive to seagrass (Jackson et al. 2001). There is mixed evidence, for example, about the preference of juvenile salmon for eelgrass habitat in estuaries (Murphy et al. 2000, but see Semmens 2008), yet references of eelgrass as foraging habitat for juvenile salmon pervade the literature (Salo et al. 1980, Simenstad et al. 1982, Webb 1991, Plummer et al. 2013). While directly sampling eelgrass blades and sediments allowed us to assess attached epifaunal and infaunal potential prey invertebrates, we recognized that juvenile salmon might not feed directly from eelgrass. Our hypothesis was that even if juvenile salmon are not persistent in eelgrass beds, and do not feed off eelgrass blades, the presence of eelgrass may promote increased prey invertebrate availability that extends beyond the eelgrass itself and into the water column. Zooplankton tows comparing prey availability over top of eelgrass beds, and over top of unvegetated sediments were conducted to help assess this possibility.

## **STUDY SPECIES**

While all Pacific salmon species are anadromous and semelparous, even within a single species, Pacific salmon display vast diversity in their biology and life histories (Healey 1991). The two salmon species focused on in this study are Chum salmon and Chinook salmon. Chum salmon are the second largest of the Pacific salmon species, with the widest habitat range; populations on the Pacific Coast of North America extend from

Southern California to the Arctic Coast (Ryall et al. 1999). Chum salmon are divided into summer runs and fall runs (DFO 1999). Summer run adults return to their natal streams in June, July, and August, to spawn in September and early October (DFO 1999). Fall runs, on the other hand, migrate in September, October, and November, to spawn from October to January (DFO 1999) with the timing of these runs varying by latitude (Salo 1991). In our study system, the Puntledge River, major runs of fall Chum salmon occur in late October to mid November (DFO 1958). Chum salmon emerge from the gravel in spring and travel almost immediately to the ocean, with peak migration in the Puntledge watershed occurring in mid May (DFO 1958). As the only major runs of summer Chum salmon in British Columbia can be found in the Bute and Knight Inlets (DFO 1999), it is likely that most of the Chum salmon caught in our study were from the fall run. Chum salmon are unique in that they have much shorter freshwater stages than other Pacific salmon (Salo 1991), with extensive marine stages lasting between 2-7 years and averaging between 3-5 years (Ryall et al. 1999).

The Chinook salmon (*Oncorhynchus tshawytscha*) is the largest of the Pacific salmon species, with spawning populations ranging from central California to Alaska on the Pacific Coast of North America (COSEWIC 2006). Across their range, different Chinook salmon populations exhibit wide variability in their life histories and spawning times (Healey 1991). Generally, Chinook salmon in the Strait of Georgia return to their natal breeding grounds between August and October, spawning in mid to late October (DFO 1999). Emergent fry hatch the following spring and exhibit one of two life histories coined by Healey (1983): stream-type and ocean-type. Stream-type Chinook salmon

spend a year in freshwater before moving to the marine environment, while ocean-type Chinook salmon migrate to saltwater environments between 1-3 months after hatching (DFO 1999). Puntledge River Chinook are predominantly ocean-type, but may be of summer run or fall run origin. Summer run Chinook migrate into their natal rivers in August/July, while fall run enter in late September/November (DFO 1999). Though these populations are genetically distinct, they enter the estuaries at the same period, and are released from the hatcheries at the same time, so we are unable to distinguish between them in this study.

Beamish and Mahnken (2001) describe a critical size, critical period hypothesis that proposes two main stages of mortality for juvenile salmon. The first stage of mortality is shortly after juvenile salmon enter the marine environment, when rapid growth reduces vulnerability to predation. The second stage of mortality occurs during the fall and winter period, when individuals that do not attain a minimum size threshold die (Beamish and Mahnken 2001). The second period of mortality is related to larger fish having more energy stored to survive periods of low food availability (Beamish and Mahnken 2001). Understanding early marine mortality is important, as there is mounting evidence suggesting that salmon return rates may be heavily impacted by their success during this early marine phase (Tomaro et al. 2012). Cohort size is established soon after ocean entry (Tomaro et al. 2012). For example, juvenile salmon abundance in the early marine period is strongly correlated with adult returns in Chinook (Murphy et al. 2013) and Coho (Beamish et al. 2010). In turn, surviving the early marine period and contributing the returning biomass is related to fish size (as reviewed by Beamish et al. 2003).

Tomaro et al. (2012) provide an overview of the growth-mortality theory, which suggests that larger fish have higher survival due to their ability to avoid predators and capture prey. Larger fish are stronger swimmers (Bainbridge 1958), which may lead to better predator avoidance abilities, and fish that grow rapidly remain susceptible to gape-limited predators for a shorter period of time (Moulton 1997). Larger fish have fewer gape size limitations (Wańkowski 1979) and are able to target larger, potentially higher quality prey items. Larger fish also have more energy stored to survive periods of low food availability (Beamish et al. 2004). This growth-mortality relationship in salmon is supported by results from numerous studies on juvenile salmon in the early marine phase. Survival of Chum salmon fry during the early marine period has been positively related to size in Alaska (Martinson 2013), Japan (Kaeriyama et al. 2007) and Canada (Farley and Moss 2009). Similar relationships have been seen for Chinook salmon in Washington (Duffy and Beachamp 2011, Tomaro et al. 2012), Coho salmon in the Strait of Georgia (Beamish et al. 2004) and the west coast of Vancouver Island (Holtby et al. 1990), and Atlantic salmon in the Baltic sea (Saloniemi et al. 2004).

Evidence suggests that rapid growth in the early marine period of juvenile salmon may be an important factor in overall survival of salmon and contribution to the spawning stock, a suggestion that is widely accepted in the scientific community (Beamish et al. 2011). The energy fish need to avoid predators is limited by foraging success (Simenstad et al. 1982). Small salmon therefore require productive feeding grounds that can readily supply food and promote growth. The productivity of juvenile salmon early marine ecosystems

and their capacity to provide prey and protection can therefore affect early marine survival and returning spawning numbers (Beamish et al. 2004, Duffy and Beauchamp 2011). Understanding how habitat characteristics such as eelgrass influence prey availability is therefore an important avenue of research.

## **JUVENILE SALMON IN ESTUARIES**

Characterized by variable salinity regimes (Miller and Sadro 2003), estuaries are often viewed as critical habitat for juvenile salmon, offering opportunities to acclimatize to saltwater environments (Neilson et al. 1985) while also playing an important role in promoting growth by supplying productive feeding grounds (Bottom and Jones 1990). Chum salmon reside in these environments for approximately one month before moving into more open waters at sizes between 50-70mm (Simenstad et al. 1982). The extent to which Chum salmon use estuaries can, however, vary across habitats (Simenstad et al. 1982). In the Nanaimo estuary, for example, Chum salmon residency ranged from 0-18 days (Healey 1979). Chinook salmon, on the other hand, are known to utilize estuaries for prolonged periods (Neilson et al. 1985, Levings and Lauzier 1991, Korman et al, 1997), residing in the Nanaimo estuary for an average of two months (Healey 1991). Neilson et al. (1985) found a marked increase in juvenile Chinook salmon growth rates upon entry to the estuarine environment. Growth rates in the Nanaimo estuary were 5.8% per day for juvenile Chinook salmon (Healey 1991), and 6% for juvenile Chum salmon (Healey 1979), with higher rates reported in other estuaries (Salo 1980). These fast growth rates indicate that productive estuaries may provide good feeding conditions for juvenile salmon (Healey 1979) during a time when growth may be critical to salmon

survival (Beamish et al. 2004). With rapid and continual development of estuarine habitat (Lotze et al. 2006) it is becoming increasingly important to understand what characteristics of estuaries make them productive feeding grounds. The purpose of this study was to determine if eelgrass is one of these important habitat characteristics promoting increased foraging opportunities for juvenile salmon.

Simenstad et al. (1982) anecdotally observed Puget Sound Pink salmon (*Oncorhynchus gorbuscha*) and Chum salmon schooling in shallow estuarine eelgrass by day and dispersing at night. Semmens (2008) posited that eelgrass habitat was used as cover after finding increased survival of juvenile Chinook salmon that preferred eelgrass habitat. A number of other studies, however, reference eelgrass as foraging habitat for juvenile salmon (Salo et al. 1980, Webb 1991, Plummer et al. 2013, Weitkamp et al. 2014).

During their early marine life, juvenile Chum salmon rely on detrital-based production in shallow marine habitats (Healey 1979) where they feed mainly on benthic organisms (Simenstad et al. 2008, Webb 1991). They are often seen along the perimeters of bays, sometimes traversing up to 2km with incoming tides to access foraging opportunities (Percy et al. 1989). Their diets are dominated by gammarid amphipods and harpacticoid copepods (Healey 1979, Simenstad et al. 1982, Salo et al. 1980). These organisms occur in higher abundances in eelgrass habitat than in unvegetated environments (Bostrom and Bonsdorf 1997). Juvenile Chinook salmon diets are generally more variable, differing between estuaries and even locations within an estuary (Healey 1991). In some locations juvenile Chinook salmon are largely zooplanktivorous, while in others, they target more benthic species (Healey 1991).

The benthic feeding habits of juvenile Chum salmon, and the reliance of juvenile Chinook salmon on the estuarine environment makes both species good candidates to investigate the importance of eelgrass for foraging of salmon. We caught juvenile Chum salmon and juvenile Chinook salmon in the Comox Estuary, and compared their diets to prey availability in zooplankton, and in epifaunal and infaunal eelgrass samples. We hypothesized that juvenile salmon diet items would be especially abundant in eelgrass samples, increasing in abundance with increased eelgrass density. We complemented this diet analysis with a stable isotope analysis in order to estimate the proportion of eelgrass invertebrates in the diets of juvenile salmon.

#### **STABLE ISOTOPE ANALYSIS**

Ecologists use natural variations in isotopic signatures (or the ratio of  $^{12}\text{C}/^{13}\text{C}$  relative to a standard ( $\delta^{13}\text{C}$ )) in the environment to help determine energy pathways in ecosystems (Post et al. 2007). Because the  $\delta^{13}\text{C}$  signatures of consumer tissues closely reflect those of their food (DeNiro and Epstein 1978, Harrigan et al. 1989), carbon isotopes can be used to determine energy sources supporting an organism (Hood-Nowotny and Knols 2007). Simenstad and Wissar (1985), for example, studied the flow of carbon in estuarine and near shore food webs in Puget Sound, estimating the diets of predators based on how closely their carbon signatures reflected those of various prey. The ratio of stable isotopes of nitrogen, on the other hand, can be used to evaluate trophic position, as the  $\delta^{15}\text{N}$  signature of consumers tend to be enriched by 3-4% relative to their diets (DeNiro and Epstein 1981).

Carbon and nitrogen stable isotopes have been widely employed to help understand food web structure in seagrass habitats (Lepoint et al. 2004). Accurately estimating trophic pathways using stable isotope analysis hinges on the ability to distinguish between carbon sources in an ecosystem (Lepoint et al. 2004). Seagrass ecosystems can be an ideal system in this respect, as seagrasses tend to be enriched in  $^{13}\text{C}$  relative to other primary producers (Hemminga and Mateo 1996), with the most common  $\delta^{13}\text{C}$  values of eelgrasses worldwide ranging between -10 and -11 (Hemminga and Mateo 1996). The enriched signature of seagrasses is related to their ability to utilize bicarbonate, which has a less depleted signature than carbon dioxide (Lepoint et al. 2004). The enrichment may also be a result of living in a submerged, and therefore carbon-limited, environment; while photosynthetic pathways in most  $\text{C}_3$  terrestrial plants tend to discriminate against  $\delta^{13}\text{C}$ , seagrasses uptake whatever is available, dampening any discrimination that would otherwise occur (Hemminga and Mateo 1996).

The distinctly enriched  $\delta^{13}\text{C}$  signature of eelgrass has been used to help determine the trophic pathways in these ecosystems (Thayer et al. 1978, Mittermayr et al. 2014). These studies focus primarily on benthic food webs and fish species that are common in eelgrass ecosystems (eg. Jaschinski et al. 2008, Ha et al. 2013). While juvenile salmon do not exclusively inhabit eelgrass ecosystems, their reliance on nearshore productivity points to the importance of eelgrass as critical habitat supporting the food webs of juvenile salmon (Plummer et al. 2013, Weitkamp et al. 2014). Using gut content analysis, studies have found juvenile Chum salmon and juvenile Chinook salmon eat a wide expanse of prey items in the marine ecosystem (Sibert 1979, King and Beamish 2000,

Duffy et al. 2010). While gut content analysis can only provide a ‘snapshot’ view of what an animal ate (Hyslop 1980), stable isotope signatures reflect consumer diets over a period of weeks to months (Post 2003, Davis et al. 2012). Additionally, gut content analysis can be biased by discrepancies between the digestibility of different organisms (Hyslop 1980), while isotope signatures actually reflect what has been assimilated from a consumer’s diet (DeNiro and Epstein 1978). We used gut content and stable isotope analysis in tandem to understand the feeding habits of juvenile Chum salmon, specifically, the proportion of eelgrass-associated benthic invertebrates in their diet.

We based the isotope analysis on three main food sources for age-0 Chum salmon in the Strait of Georgia; terrestrial insects, pelagic crustaceans, and benthic invertebrates (King and Beamish 2000). As the relative contribution of prey items to predator isotope ratios can become ambiguous with the consumption of multiple carbon sources (Simenstad and Wissmar 1985). We expected that terrestrial insects would be the most depleted source, reflecting the  $\delta^{13}\text{C}$  signature of supra-littoral plants relative to the enriched marine signature of zooplankton (Romanuk and Levings 2005). Benthic invertebrates in eelgrass, however, would be reliant on eelgrass carbon (Ha et al. 2013) and therefore would be more enriched than zooplankton. We hypothesized that juvenile Chum salmon would have more depleted  $\delta^{13}\text{C}$  signatures relative to zooplankton and insects, reflecting their benthic feeding habits in eelgrass ecosystems. Having site-specific knowledge of juvenile Chum salmon and juvenile Chinook salmon diets in the Comox Estuary will aid in understanding important near-shore habitat features for Puntledge River salmon stocks.

## SUMMARY

Knowledge of how juvenile salmon interact with near-shore habitat characteristics, such as eelgrass beds, gives insight in to how habitat development and changes to the near-shore environment can affect these species. Such information allows managers to better understand the implications of habitat loss, degradation, or habitat restoration for salmon survival. We hope that a combination of epifaunal and infaunal eelgrass samples, and zooplankton samples would help understand how changes in eelgrass habitat can affect prey availability for juvenile salmon. We analyzed the diets of juvenile Chum salmon and Chinook salmon, and assessed stable isotopes in juvenile Chum salmon to help determine the importance of eelgrass in providing foraging opportunities for juvenile salmon. Employing a combination of stable isotope analysis and gut content analysis helped overcome some of the drawbacks of traditional gut content analysis (Post 2003), while still offering an understanding of consumer diet down to a greater taxonomic resolution than isotope analysis would have allowed for (Davis et al. 2012).

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## **Chapter 2: The Importance of Eelgrass as Foraging Habitat for Juvenile Salmon**

### **GENERAL PROJECT INTRODUCTION**

Pacific salmon (*Oncorhynchus* spp.) play a primary role in the ecology of marine and terrestrial systems, supporting marine food webs (Adams et al. 2016), and facilitating nutrient transport into terrestrial ecosystems, with important consequences for numerous plant and wildlife species (Hilderbrand et al. 2004). Pacific salmon are also an economic resource (Beamish et al. 2003), and a cultural foundation for First Nations' communities along the coast of British Columbia (Gerwing and McDaniels 2006). Despite the economic, cultural, and ecological importance of this iconic genus, the multitude of natural and human-induced challenges Pacific salmon face throughout their life histories are still poorly understood (Beamish et al. 2003). For example, very little is known about what caused the abrupt decline of Chinook salmon in the Strait of Georgia in the 1990s (Heard et al. 2007).

While decreased salmon returns have largely been attributed to mortality in the early marine phase, we have little understanding of this phase of Pacific salmon life histories (Beamish et al. 2003). The critical size hypothesis suggests that rapid salmon growth during the early marine phase strongly correlates with increased survival to adulthood and contribution to the spawning stock (Beamish and Mahnken 2001). Among other studies, survival of Chum salmon fry during the early marine period has been positively related to size in Alaska (Martinson 2013), Japan (Kaeriyama et al. 2007) and Canada

(Farley and Moss 2009). Similar relationships have been seen for Chinook salmon in Washington (Duffy and Beachamp 2011, Tomaro et al. 2012), Coho salmon in the Strait of Georgia (Beamish et al. 2004) and the west coast of Vancouver Island (Holtby et al. 1990), and Atlantic salmon in the Baltic sea (Saloniemi et al. 2004). Larger fish have more energy reserves to use during subsequent periods of low food availability (Beamish et al. 2004) and growth reduces vulnerability to predation in early marine residence (Moulton 1997). This critical size hypothesis predicts that the productivity of ecosystems is important for juvenile salmon and that the capacity of these ecosystems to provide prey can affect early marine survival and returning spawning numbers (Beamish et al. 2004). Duffy et al. (2010) hypothesized that decreased survival of salmon in Puget Sound may be a reflection of near-shore habitat degradation and Beamish et al. (2003) recommended an investigation into the role of estuaries as nursery habitat for juvenile salmon.

Estuaries are productive near-shore habitats, where salmon display high growth rates (Healey 1991, Nielson et al. 1985). Magnusson and Hilborn (2003) were the first to study the importance of productive estuaries to salmon survival directly. They found that Chum salmon and Chinook salmon fry introduced into healthy estuaries had higher survival rates compared to fry introduced to degraded estuaries. We chose to study the function of estuarine ecosystems for juvenile salmon ecology using eelgrass as a focal habitat due to the proposed importance of eelgrass for juvenile salmon (Fujiwara et al. 1997).

Eelgrass habitat in Canada is recognized as an Ecologically Significant Species, creating preferential habitat for a number of taxa, providing resources and protection for its

inhabitants, and having a disproportionate influence on ecosystem structure and function (DFO 2009). Increasing coastal human populations and near-shore development, however, put increased pressure on the near-shore habitats where eelgrass is found (Johannessen and Macdonald 2009). As well as posing higher risk of physical disturbance with increasing human population density, anthropogenic activities can also have indirect effects that threaten the integrity of eelgrass habitat. Sediment runoff resulting from forestry practices (Sheridan et al. 1999), and algal blooms resulting from ocean climate changes (Johannessen and Macdonald 2009), can decrease light penetration into the water column, which is a limiting factor for eelgrass range and survival (reviewed in Kim et al. 2015). Increasing water column depth due to sea level rise is also predicted to shade out large portions of extant eelgrass beds (Plummer et al. 2012). In the face of large-scale eelgrass habitat destruction in many Pacific estuaries (eg. Tallis et al. 2009, Thom et al. 2012), impending future losses due to climate change (Shaughnessy et al. 2012), as well as drastically diminished returns of Pacific salmon across the West Coast of Canada (Noakes et al. 2000), increased knowledge on how eelgrass habitat affects juvenile salmon will have important management implications.

The goal of this study was to determine if eelgrass beds offer near-shore foraging habitats for juvenile salmon. The study had two key objectives:

- 1) To understand how eelgrass density and form influence juvenile salmon prey, in terms of both prey abundance and community structure; and
- 2) To quantify the contribution of eelgrass invertebrates in juvenile salmon diets.

We sampled eelgrass to obtain metrics of eelgrass habitat (eelgrass shoot density, biomass, shoot length, shoot width, surface area, and epiphyte biomass) and potential salmon prey invertebrates in epifaunal and infaunal eelgrass samples across a gradient of eelgrass density. We hypothesized that eelgrass would host a variety of infaunal and epifaunal prey that increased in abundance with eelgrass shoot density, and that the species assemblages in sparse eelgrass would have higher compositions of sediment associated species, while assemblages in dense eelgrass would have higher compositions of eelgrass associated species. We also conducted zooplankton tows to compare prey availability in the water column over eelgrass and unvegetated sediment. We hypothesized that the presence of eelgrass would influence the zooplankton invertebrate communities over top of eelgrass habitat, and that the tows over eelgrass would display increased abundances of eelgrass-associated invertebrates. We compared eelgrass prey invertebrates to the diets of juvenile Chum salmon, with the hypothesis that juvenile Chum salmon utilized invertebrate communities found in eelgrass habitat.

We complemented our diet analysis with stable isotope ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) analysis of juvenile Chum salmon and their potential diet sources (epifaunal eelgrass invertebrates, zooplankton, and terrestrial invertebrates). The  $\delta^{15}\text{N}$  signatures of consumers tend to be enriched by 3-4‰ relative to their diets, and can be used as an indication of trophic level (DeNiro and Epstein 1981), while the  $\delta^{13}\text{C}$  signatures of consumer tissues closely reflects those of their food source (DeNiro and Epstein 1978, Harrigan et al. 1989). Using these

stable isotopes in tandem can help trace carbon pathways in an ecosystem (Lepoint et al. 2004).

We expected that terrestrial insects would be the most depleted potential carbon source, reflecting the  $\delta^{13}\text{C}$  signature of supra-littoral plants, while zooplankton would display a more enriched marine signature (Romanuk and Levings 2005). We expected benthic invertebrates in eelgrass, however, to be reliant on eelgrass carbon (Ha et al. 2013), which has a distinctly enriched  $\delta^{13}\text{C}$  signature relative to terrestrial and marine sources (Mittermayr et al. 2014). Finally, we hypothesized that juvenile Chum salmon would have more depleted  $\delta^{13}\text{C}$  signatures relative to zooplankton and insects, reflecting their benthic feeding habits in eelgrass ecosystems. While gut content analysis can only provide a ‘snapshot’ view of what an animal ate (Hyslop 1980), stable isotope signatures reflect what an animal ate over a period of weeks to months (Post 2003, Davis et al. 2012). Additionally, gut content analysis can be biased by discrepancies between the digestibility of different organisms (Hyslop 1980), while isotope signatures actually reflect what has been assimilated from a consumer’s diet (DeNiro and Epstein 1978). Employing a combination of stable isotope analysis and gut content analysis can overcome some of the drawbacks of traditional gut content analysis (Post 2003), while still gaining an understanding of consumer diet down to a greater taxonomic resolution than isotope analysis itself allows for (Davis et al. 2012).

All sampling was completed over a 12 day period in May, 2015 to ensure that all samples for stable isotope analysis were taken over the same time frame. We conducted a smaller,

supplementary survey in May, 2016 to further assess eelgrass invertebrates across a larger eelgrass shoot density range, and to compare the diets of juvenile Chinook salmon to prey availability in eelgrass habitat.

Knowledge of how juvenile salmon interact with near-shore habitat characteristics, such as eelgrass beds, gives insight in to how habitat development and changes to the near-shore environment can affect these species. Such information allows managers to better understand the implications of habitat loss, degradation, or habitat restoration for salmon survival. This information is especially important given the depressed populations of many salmon populations in the Salish Sea (Beamish et al. 2003), and the continued loss of eelgrass habitat (Short et al. 2010).

## **METHODS**

### *Study system*

The Comox Estuary is located on the east coast of Vancouver Island, British Columbia, Canada, in the northern region of Baynes Sound within the Salish Sea (Figure 1). The 1470 ha estuary is relatively shallow (Morris et al. 1979), with eelgrass covering approximately 400 ha and existing as a largely continuous patch within which there are varying densities (Project Watershed 2015). Much of the mapped eelgrass is intertidal and accessible at low tide from shore (Project Watershed 2015). The Tsolum and Puntledge Rivers, which connect to form the Courtenay River on the outskirts of the city

of Comox, deliver an average of 57.3 cm/s of freshwater into the estuary (Morris et al. 1979).

This river system is historical rearing habitat for all seven species of Pacific salmon (Trites et al. 1996). The Puntledge River Chum salmon are part of the Mid Vancouver Island geographic unit, an abundant commercial stock that made up 20% of total Chum salmon catches in British Columbia between 1990-1996 (Ryall et al. 1999). The Chinook salmon in the Puntledge River are the focus of conservation efforts to reduce the impacts of a BC Hydro dam constructed in the 1950s (Trites et al. 1996). The Puntledge River hatchery releases 3-6 million Chum salmon (Yurk and Trites 2000), and over 2 million Chinook salmon (Puntledge hatchery, Comox BC- unpublished data) annually from its tributaries. Average escapement between 2005-2015 was over 76,000, and approximately 4500 for Chum salmon and Chinook salmon respectively (Puntledge hatchery, Comox BC- unpublished data). Juvenile salmon are typically present in the estuary from April-August (Tryon 2011). The accessibility of eelgrass and the abundance and importance of salmon in the Puntledge River made the Comox Estuary an ideal location to test our hypotheses.

### *Field Methods*

#### Prey Availability – Epifaunal eelgrass sampling

To assess the relationship between eelgrass and epifaunal prey invertebrates, in May 2015, 40 sampling points, separated by at least 50m, were randomly generated over potential eelgrass areas. Eelgrass was sampled at each point using a 0.25m<sup>2</sup> quadrat.

Total shoots were counted in the entire quadrat, and shoots in  $\frac{1}{4}$  ( $0.0625\text{m}^2$ ) of the quadrat were counted, harvested, and stored in 80% ethanol for analysis of eelgrass metrics and invertebrate abundance. To encourage regrowth of sampled eelgrass, the shoots were cut just above the meristem, leaving the entire rhizome intact. If sampling points were immersed in seawater at the time of sampling, water depth was recorded to control for the possibility of invertebrates either escaping capture by migrating from eelgrass into the water column, or entering eelgrass from the sediments through the water column. Using the same collection methods, in May 2016, we sampled an additional 30 intertidal sampling points to cover a wider expanse of eelgrass shoot density.

#### Prey Availability – Infaunal eelgrass sampling

To assess the relationship between eelgrass and infaunal prey invertebrates, an 8cm diameter, 10cm deep sediment core was collected for a subsample of 29 quadrats in May 2015. The sediment cores were stored in 80% ethanol for infaunal prey invertebrate abundance and sediment size analysis.

#### Prey Availability - Zooplankton sampling methods

To determine if the presence of eelgrass would influence the availability of prey in the water column, zooplankton tows were conducted in the Comox Estuary in May, 2015. Twenty-four tows were conducted in total, 12 over eelgrass, and 12 sites over unvegetated sediment (Figure 1). These tows allowed for the comparison of eelgrass-associated invertebrate availability between vegetated and unvegetated sites in the estuary. An additional 12 tows were conducted in unvegetated sites outside of the

estuary. These outer estuary tows were conducted to control for the fact that finding eelgrass-associated prey in both vegetated and unvegetated inner estuary tows would make it difficult to determine if the invertebrates were coming from eelgrass and simply transported to unvegetated sites via seawater movement, or if the invertebrates were present in the water column despite the absence of eelgrass. If eelgrass-associated invertebrates were present in all tows within the estuary, but not present in outer estuary tows, we would conclude that any eelgrass-associated invertebrates found in the inner estuary tows likely migrated from eelgrass habitat. Outer estuary tows were conducted just north of the Comox Estuary to avoid influence from estuarine water flowing south (Morris et al. 1979) (Figure 1).

Tow sites were separated by a minimum of 50m and chosen haphazardly, as maintaining a position generated randomly was impractical with a small vessel. Bottom depth was measured at the beginning of each tow, along with salinity and temperature at the surface and every 1m until ocean bottom or until a depth of 5m was reached. The plankton tow net was made of 200 $\mu$ m white Nitex mesh with a diameter of 0.5m, tapering to 10cm at its base. A TSK flow meter suspended in the mouth of the net was used to measure the volume of seawater sampled so it could be corrected for. The net was suspended approximately 1m below the water, with the aim of sampling just above the height of any eelgrass present. The net was towed towards the nearest shoreline for two minutes at a speed of approximately 1.5 knots. All inner-estuary tows were monitored visually to ensure eelgrass was present or absent along the entire tow length. After two minutes of towing, the bottom 1.5m of the net was dipped three times into the water and brought up

swiftly to encourage zooplankton into the codend. The lower 0.5m of the net was then rinsed with freshwater four times into the detachable codend. Zooplankton collected in the codend were kept cold in a cooler, and later preserved in 4% formaldehyde solution for abundance analysis.

The plankton tows were conducted from the 10<sup>th</sup> to the 13<sup>th</sup> of May 2015, from 09:00-14:00. Most benthic invertebrates, however, reside in the substrate during the day and migrate into the water column after dark (Robertson and Howard 1978, Alldredge and King 1980). To address the possibility that eelgrass-associated invertebrates only migrated into the water column at night, additional zooplankton sweeps were conducted in May 2016. Though the same net and towing methods were used, 2016 tows are referred to as sweeps as information on the depth, salinity, temperature, and seawater volume sampled was not collected. Within the estuary, six invertebrate sweeps over eelgrass vegetated sites, separated by at least 50m, were conducted between 15:00 and 18:00. Six sweeps were repeated over the same sites between 21:00 and 23:00 on the same day to compare availability of eelgrass-associated invertebrates in the water column between the day and the evening.

#### Juvenile salmon diet - Juvenile salmon sampling

Juvenile salmon were collected for diet content analysis in order to determine if they were utilizing eelgrass invertebrate communities as prey. Fish were captured only after animal care protocols from the Animal Care Committee of the University of Victoria and sampling permits from the Department of Fisheries and Oceans were granted. After

multiple test tows around the estuary in 2015, two locations were chosen as beach seine sampling sites (Figure 1), as they were the only locations where juvenile Chum salmon were caught. On May 12<sup>th</sup> and 13<sup>th</sup>, 2015, between 9:30 and 13:00, juvenile Chum salmon were caught in a total of 5 tows using a 10x2m beach seine, which tapered to 1m in height, with 3mm mesh. The majority of the net was dragged onto shore, leaving the middle portion in the water to form a pseudo-codend to avoid taking fish onto land. All non-salmonids or non-target salmonids were immediately returned to the water. Salmon were transferred to seawater holding tanks, with no more than 20 juveniles per 12L to minimize stress. Juvenile Chum salmon were identified to species and then euthanized using an overdose of MS222 in a seawater bath (300mg/L). The euthanized salmon were weighed to the nearest 0.1g and their fork lengths were recorded to the nearest 0.1mm. The spinal cord of each fish was then broken to ensure mortality according to the University of Victoria animal care protocols, and fish were immediately placed on ice. Within 4 hours, stomachs were dissected from each fish, and preserved in 80% ethanol for stomach content analysis. In 2016, between 10:30 and 12:30 on May 10<sup>th</sup>, 2016, juvenile Chinook salmon were caught at the same sites and processed using the same methodology.

#### Juvenile salmon diet - Isotope sample collection

In 2015, samples of juvenile Chum salmon and their potential diet sources (epifaunal eelgrass invertebrates, zooplankton, and terrestrial invertebrates) were collected for stable isotope analysis to determine the importance of these sources in the diets of juvenile Chum salmon. At ten of the most heavily vegetated eelgrass sampling points, eelgrass

from an additional 0.0625m<sup>2</sup> quadrat was harvested to collect epifaunal eelgrass invertebrates for stable isotope analysis. Zooplankton samples were collected via two five-minute tows outside of the estuary using the same zooplankton tow net described above (Figure 1). For freshwater/terrestrial invertebrates, the zooplankton tow net was halfway submerged and suspended in the Courtenay River (Figure 1). Rocks, logs, and sediment were disturbed along a 0.5m transect for 2m ahead of the suspended net to flush out stream invertebrates. Invertebrates were collected from the codend. The net was then resuspended 10m ahead of the original site for five minutes, allowing for the collection of freshwater/terrestrial invertebrates naturally flowing down the river. The same process was repeated upriver (Figure 1). All collected samples were kept cool and frozen at -18°C within four hours. For juvenile Chum salmon, after the stomachs had been dissected, the remaining carcasses were also frozen at -18°C. We expected that terrestrial insects would be the most depleted source, reflecting the  $\delta^{13}\text{C}$  signature of supra-littoral plants relative to the enriched marine signature of zooplankton (Romanuk and Levings 2005). Benthic invertebrates in eelgrass, however, would be reliant on eelgrass carbon (Ha et al. 2013) and therefore would be more enriched than zooplankton. We hypothesized that juvenile Chum salmon would have more depleted  $\delta^{13}\text{C}$  signatures relative to zooplankton and insects, reflecting their benthic feeding habits in eelgrass ecosystems.

### *Lab Processing*

Prey availability - Eelgrass epifaunal prey invertebrates

Eelgrass blades were rinsed and scraped of invertebrates and epiphytes. Three of the

tallest shoots were measured to the nearest mm to get average shoot length of the quadrat, ignoring the tallest 20% to get the best measure of the average length of the eelgrass (Short 2001). The same blades were measured above the meristem to get average shoot width. The eelgrass was spread out on trays to dry for 48 hours in a 60°C drying oven and then weighed to the nearest 0.0001g to estimate eelgrass biomass in each quarter quadrat. Epiphyte biomass was dried and weighed to the nearest 0.0001g. Eelgrass invertebrates were separated into the lowest taxonomic group feasible with time constraints (polychaete worms, snails, harpacticoid copepods, limpets, barnacles, crabs, mussels, mites, barnacle cyprids, shrimp zoea, decapod shrimp, ostracods, crab zoea, crab megalopae, euphausid zoea, cladocerans, corophid amphipods, tanaids, small (<1cm) and large (>1cm) isopods, cumaceans, gammarid amphipods, other senticaud amphipods, other amphipods, and dipterans). Data are expressed in terms of abundance/0.0625m<sup>2</sup>.

#### Prey availability - Eelgrass infaunal invertebrates

Sediments were separated into size classes by sieving them through 500µm, 250µm, 125µm, and 63µm mesh. The sediments were carefully examined to ensure complete removal of infaunal invertebrates after a gentle wash. Sediments were dried at 60 °C for 48 hours and weighed to the nearest 0.1g. Infaunal invertebrates were then identified and counted. Data were expressed in terms of abundance/sediment core.

#### Prey availability - Zooplankton

For the 2015 invertebrate tow samples, large items in the zooplankton, such as medusae, larval fish, and large decapod shrimp, were removed from the sample and enumerated

before any sample splitting occurred. After splitting, remaining zooplankton invertebrates were then identified and counted. Data were expressed in terms of abundance/m<sup>3</sup>. As the 2016 invertebrate sweeps were conducted only to determine if eelgrass species migrated into the water column, only species presence/absence was recorded for these sweeps.

#### Juvenile salmon diet - Stomach contents

Total weight and stomach lining weight of juvenile salmon stomachs from the esophageal opening to the pylorus (Godin 1981) were recorded to the nearest 0.0001g. Invertebrates were analyzed using a dissecting microscope and separated into the lowest taxonomic resolution feasible. Each prey taxon was counted, blotted on absorbent paper four times on each side to dry individuals, and weighed to the nearest 0.0001g (Brodeur et al. 2007). Similar to Landingham et al. (1998), a qualitative estimate of digestion was recorded on a scale of 1-3 (1 = fresh, no digestion, 3 = heavily digested).

Because juvenile Chum salmon exhibit size selective predation on copepods (Chebanova et al. 2015), the size of consumed copepods was compared to those available in epifaunal and infaunal eelgrass samples and in zooplankton. Copepod length measurements were taken for the first 5-10 copepods encountered in each sample. In all samples except for zooplankton, the first 5-10 copepods encountered were harpacticoid copepods. Because harpacticoid copepods in zooplankton samples were rare (see Results) cyclopoid copepods lengths for zooplankton samples were measured instead. All copepods were measured to the nearest 0.1µm from the tip of the head to the insertion of the caudal setae (Lawrence et al. 1987).

## Juvenile salmon diet – Stable isotopes

Dorsal muscle tissue was dissected from a random sample of thawed juvenile Chum salmon, dried for 48 hours at 50°C, ground to a homogenous powder, and stored on silica gel desiccant in a sealed vial until packed and shipped for isotope analysis (Heck et al. 2008). For eelgrass samples, shoots were scraped of epiphytes and invertebrates and rinsed in distilled water before drying. Only healthy (green) eelgrass blades above the meristem were used for stable isotope analysis. All invertebrates were picked from epiphytes before epiphytes were ground. Eelgrass invertebrates collected included amphipods, polychaete worms, copepods, and tanaids, but copepods and tanaids were excluded from the isotope analyses as they did not meet minimum weight requirements. Individual polychaete worms were used for isotope analysis, while amphipods were pooled in groups of 5-10 before being ground to overcome weight limitations. Zooplankton samples were rinsed of particulate organic matter with distilled water over 500µm mesh, which left mostly calanoid copepods and crustacean nauplii. Zooplankton was analyzed in bulk, with each dried sample weighing between 3-5mg and composed of 100+ individuals. Stream invertebrates collected consisted of mayflies and gammarid amphipods. The gammarid amphipods all came from the lower section of the river, which was influenced by tidal action.

Samples were run on a Thermo Delta IV Isotope Ratio Mass Spectrometer (University of Victoria). Carbon and nitrogen isotope signatures are expressed using delta notation as parts per thousand (‰) relative to the standard according to the following equation:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000\%$$

Where  $R_{\text{sample}}$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  or  $^{15}\text{N}/^{14}\text{N}$  in the sample, and  $R_{\text{standard}}$  is the ratio of  $^{13}\text{C}/^{12}\text{C}$  in Pee Dee Belmenite or of  $^{15}\text{N}/^{14}\text{N}$  in atmospheric nitrogen.

### *Data Analysis*

#### Prey availability - Eelgrass epifaunal prey invertebrates

Data from 2015 and 2016 were pooled for all eelgrass invertebrate analyses to allow for assessment of invertebrates across the widest range of eelgrass density. For categorization purposes, eelgrass shoot density per  $\text{m}^2$  was calculated from the number of shoots in the  $0.0625\text{m}^2$  quadrats, and samples were lumped into eelgrass shoot density categories of low (0 - 49 shoots/ $\text{m}^2$ ), medium (50 - 99 shoots/ $\text{m}^2$ ), and high (100+ shoots/ $\text{m}^2$ ) (Webster et al. 1998). Eelgrass variables, such as average shoot length and width, were calculated for each eelgrass shoot density category for each year to display differences in eelgrass variables between years and eelgrass shoot density categories. Limpets, barnacles, large isopods, and snails were all found in the eelgrass epifaunal samples, but were excluded from the analysis because they were patchily distributed and considered either too calcareous or too large to be juvenile salmon diet items. Together these items constituted 20.6% of the total abundance of epifaunal invertebrates (Table S1). Because the focus was specifically on eelgrass-associated epifaunal prey invertebrates, planktonic species such as crab megalopae, shrimp zoea, cyprid barnacles, decapod shrimp, euphasid larvae, cladocerans, and ostracods were also excluded from the analysis (Suthers and Rissik 2009, Feller and Kaczinski 1975), leaving polychaete worms, harpacticoid copepods,

tanaids, isopods, cumaceans, and amphipods. The excluded planktonic invertebrates represented 0.2% of the total abundance (Table S1).

A generalized linear model was used to assess how eelgrass epifaunal prey invertebrate abundance was affected by eelgrass variables. Potential explanatory variables included epiphyte biomass/shoot (g), eelgrass biomass (g), shoot density/0.0625m<sup>2</sup> quadrat, shoot density in the entire 0.125m<sup>2</sup> quadrat, eelgrass surface area (cm<sup>3</sup>) in the 0.0625m<sup>2</sup> quadrat, and variables such as year and water depth. First, Pearson correlations between each pair of potential explanatory eelgrass variables were calculated in order to identify any collinearity. To avoid multicollinearity in the generalized linear model, only eelgrass shoot density/0.0625m<sup>2</sup> was included (since eelgrass biomass, eelgrass density/0.0625m<sup>2</sup>, shoot density in the entire 0.125m<sup>2</sup>, and eelgrass surface area were correlated; see Results). Eelgrass shoot density is a quick and inexpensive parameter to measure (Fonseca et al. 1990) and is commonly used to assess eelgrass habitat damage or restoration (eg. Wright 2014, Yi et al. 2014, Eriander et al. 2016). Other studies have also supported the hypothesis that eelgrass shoot density is an important predictor of epifaunal invertebrate abundance (Fonseca et al. 1990, Hovel et al. 2016).

In order to choose the appropriate distribution for the generalized linear model (GLM), a histogram of total epifaunal prey invertebrate abundance was examined for skew and zero inflation (Figure S1). Since a Poisson distribution provided a poor fit to the data due to overdispersion, a GLM with a negative binomial distribution and a log link was employed (Bliss and Fisher 1953). Total epifaunal prey invertebrate abundance was

modeled against eelgrass shoot density/0.0625m<sup>2</sup> (eelgrass shoot density), epiphyte biomass/shoot, year, water depth, and an interaction between epiphyte biomass/shoot and eelgrass shoot density. Continuous predictor variables were standardized by subtracting the mean value from each observation and dividing each observation by the standard deviation (Schielezeth 2010). Standardization allows for a more direct comparison of the strength of the coefficients for each explanatory variable (Schielezeth 2010).

Standardization also allows for each coefficient to be calculated for each variable with all other variables held at their mean values instead of at zero (Schielezeth 2010). Computing coefficients with other variables held at their means instead of at zero was more representative of the ecology of eelgrass habitat, as it is uncommon to have eelgrass shoots with 0 epiphyte biomass, and it is impossible to have any epiphyte biomass when no eelgrass shoots are present. Additionally, using a standardized value of year meant the intercept for the model was calculated over an average of both years, which was appropriate for our data as we were not particularly interested in the differences between years (Schielezeth 2010). A stepwise procedure was used to exclude non-significant input variables, and a likelihood ratio test was performed to assess the significance of categorical variables. A likelihood ratio test confirmed the better fit of a negative binomial distribution compared to a Poisson distribution. Plots of residuals against fitted values, and residuals against leverage were examined to ensure the model fit and to identify outliers. We repeated the analysis excluding a potential outlier to ensure the model output was robust. To further justify the exclusion of other eelgrass variables besides shoot density/0.0625m<sup>2</sup> in model, the final model with year and epiphyte biomass

was run with all possible combinations of covariates, eelgrass density/0.0625m<sup>2</sup>, shoot density in the entire 0.0125m<sup>2</sup>, and eelgrass surface area in the 0.0625m<sup>2</sup> quadrats.

Spatial autocorrelation in prey invertebrate abundance was assessed with Moran's *I* statistic (Moran 1950), using the following equation:

$$I = \frac{n}{(n-1)S^2h} \sum_{i=1}^n \sum_{j=1}^n h_{ij} (Y(t_i) - \bar{y})(Y(t_j) - \bar{y})$$

Where *n* = sample size, *S*<sup>2</sup> = variance in prey invertebrate abundance between samples, *h* = the sum of the inverse distance weights, *Y*(*t*<sub>*i*</sub>) and *Y*(*t*<sub>*j*</sub>) = prey invertebrate abundances at any two points,  $\bar{y}$  = the mean prey invertebrate abundance. Spatial autocorrelation was indicated if the computed Moran's *I* was significantly different than the expected value of *I* under the null hypothesis:

$$I = \frac{-1}{(n-1)}$$

To assess the effect of eelgrass shoot density on invertebrate community composition, a constrained analysis of principal coordinates (CAP) was used to visualize differences between invertebrate communities across low, medium, and high eelgrass densities. A CAP is an ordination technique that allows for the use of Bray-Curtis distances (Legendre and Anderson 1999). Bray-Curtis distance is the preferred measurement used for ecological data (Legendre and Gallagher 2001) because only joint species presence is considered when calculating a distance matrix between sampling points (Legendre and Legendre 2012). Joint absences of rare species, which are common in ecological data, therefore do not imply similarity (Legendre and Legendre 2012). Each invertebrate count was divided by the maximum count for that species to standardize invertebrate

abundances. This standardization method computed relative abundances of species, which allowed for rare and abundant species to be equally weighted (ter Braak et al. 1995). For the original analysis, eelgrass shoot density, eelgrass biomass, surface area, year, and water depth were included as explanatory variables. An ANOVA-like permutation test was performed to determine if the CAP model was significant, and to determine which explanatory variables were significant predictors of eelgrass prey invertebrate communities. Backward stepwise regression was performed to exclude non-significant variables. Variance partitioning was then used to determine how much of the variation in eelgrass epifaunal prey invertebrate communities was explained by each significant variable. Autocorrelation was assessed using a Mantel test with 1000 permutations.

#### Prey availability - Eelgrass infaunal prey invertebrates

Planktonic and calcareous species made up <1% of total abundance (Table S4) and were excluded from the analyses as per the eelgrass epifaunal prey invertebrate methods. To quantitatively assess how infaunal prey invertebrate abundance was affected by eelgrass density, infaunal prey invertebrate abundance was modeled as a function of eelgrass shoot density, sediment depth, and percent fine sediment using a GLM. Percent fine sediment was calculated as the sum of the dry weight of sediment particles <125 $\mu$ m divided by total sediment weight. The percent of fine sediment was included in the model because infaunal invertebrate abundances and composition can be influenced by sediment size (Coblentz et al. 2015). In order to choose the appropriate distribution for the generalized linear model, a histogram of total infaunal invertebrate abundance was

examined for skew and zero inflation (Figure S3). After again finding a poor fit to the data with a Poisson model, a GLM with a negative binomial distribution and a log link was chosen, with a likelihood ratio test to confirm better fit. Total infaunal prey invertebrate abundance was modeled as a function of eelgrass shoot density, sediment depth, and percent fine sediment. Plots of residuals against fitted values and residuals against leverage were examined to ensure the model fit and to identify. Spatial autocorrelation was again assessed using Moran's *I*.

To further assess potential differences in infaunal invertebrate communities across eelgrass density, a CAP was used to visualize the differences between infaunal invertebrate communities across low, medium and high eelgrass shoot density categories, with eelgrass density, sediment depth, and fine sediments as explanatory variables. The same procedure for the CAP on eelgrass epifaunal prey invertebrate communities was followed.

#### Prey availability - Zooplankton

The presence of eelgrass-associated invertebrates in zooplankton was assessed in 2015 invertebrate tows and in the 2016 invertebrate sweeps. For the 2015 tows, a CAP was used to visualize differences between zooplankton communities overtop of eelgrass, unvegetated habitat, and outside of the estuary, with salinity, temperature, volume of seawater sampled, bottom depth, and tow type as explanatory variables.

## Juvenile salmon diet - Stomach contents

To determine if juvenile salmon diets were adequately described, species accumulation curves were plotted for juvenile Chum salmon and Chinook salmon diets using a random ordering technique with 999 permutations. Diets were considered adequately sampled if a derivative of  $<0.05$  was reached at the sample size collected, indicating a low probability of finding additional prey species with increased fish sample size (Cook and Bundy 2010).

The relative importance of each invertebrate group by count was visually assessed in juvenile Chum salmon and juvenile Chinook salmon using a stacked bar graph. Because invertebrate group weight data were available for juvenile Chum salmon, the relative importance of each invertebrate group by weight was also visually assessed using a stacked bar graph (Figure S4). The proportion of total abundance of benthic eelgrass-associated invertebrates, planktonic invertebrates, and terrestrial invertebrates was calculated. Benthic eelgrass associated species included harpacticoid copepods, gammarid amphipods, corophid amphipods, cumaceans, polychaete worms, and tanaids. Planktonic invertebrates included sea snails, barnacle cyprids, shrimp zoea, crab megalopae, crab zoea, decapod shrimp, ostracods, euphasid larvae, cladocerans, juvenile fish, calanoid copepods, crustacean nauplii, and hyperid amphipods. Terrestrial invertebrates included insects and ants.

To investigate the factors influencing the importance of eelgrass associated invertebrates in juvenile salmon diets, a CAP was performed on juvenile salmon diets with juvenile

salmon metrics, including fork length, fish condition (see below), gut fullness (see below), tow number, and level of digestion as explanatory variables. Fork length was included in the model, as fish length can affect the size of prey items targeted by juvenile salmon (eg. Duffy et al. 2010), and could therefore influence the presence of larger eelgrass prey invertebrates in the diet.

To calculate relative weight (condition), first length–weight equations were calculated as:

$$\log(W_i) = \log(a) + b\log(L_i) + \epsilon_i$$

Where  $W$  = fish weight (g),  $a$  = intercept,  $b$  = slope,  $L$  = fish length (mm), and  $\epsilon_i$  = is the multiplicative error term for the  $i$ th fish. The resulting length-weight equations for juvenile Chum salmon and juvenile Chinook salmon were used to predict the expected weight (standard weight ( $W_s$ )) of each fish at their given length, and condition was determined for each fish with the following formula:

$$W_r = W/W_s * 100$$

Where  $W_r$  is relative weight,  $W$  is the true weight of the fish (g), and  $W_s$  is the standard weight. Relative weight (condition) is an index of fish condition that displays how well an individual fish performs compared to other fish caught (Tesch 1968). Condition was included in the model as it may influence competition and what resources a fish is able to acquire (Abbott et al. 1985). Gut fullness can be used to assess how easy prey are captured by fish, and may therefore be an indication of prey availability in the estuary (Healey 1991). Gut fullness was included in the model, because prey availability can influence the types of food targeted by juvenile salmon (Tiffan et al. 2014). Gut fullness was calculated by dividing total gut content weight (total stomach weight - stomach

lining weight) by total fish weight. To control for the fact that some invertebrates are digested at different rates than others (Macdonald et al. 1982), the level of digestion (1-3) was included as a categorical variable. Because fish from the same tows may be exposed to the same prey availability, the effect of tow was conditioned out.

As juvenile Chum salmon display size selectivity for copepods (Feller and Kaczynsky 1975), length of harpacticoid copepods in juvenile Chum salmon diets was compared to the length of copepods found in epifaunal and infaunal eelgrass prey invertebrate samples and zooplankton tows. In addition, to assessing selectivity, copepods lengths were compared across the potential sources of prey to determine if the potential prey pools offered the same size range of copepods that was targeted by juvenile Chum salmon. A Levene's test was used to ensure copepod length data met the assumption of equality of variance, and an ANOVA was used to test for significant differences in average copepod length in juvenile Chum salmon, epifaunal and infaunal eelgrass samples, and zooplankton samples. Post hoc comparisons between different samples were carried out with a Tukey's honest significant difference test (Tukey's HSD).

#### Juvenile salmon diet – Stable isotopes

Stable isotope analysis was used to estimate the contribution of epifaunal eelgrass prey invertebrates, zooplankton invertebrates, and terrestrial insects to the diets of juvenile Chum salmon. As it was difficult to obtain sufficient weight of dried insects for stable isotope analysis, literature values of -27.0 for  $\delta^{13}\text{C}$  and 6.0 for  $\delta^{15}\text{N}$  were used as estimates for terrestrial invertebrates. These estimates are mean values of black flies from

four sites in the Miramichi River system in New Brunswick (Doucett et al. 1996). Values from Doucett et al. (1996) offered an estimate for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of dipteran flies, which were the most common terrestrial invertebrate found in juvenile Chum salmon diets in this study (See Results). The estimate served as a good proxy for terrestrial insects in this study because the average  $\delta^{13}\text{C}$  of terrestrial plants (-29.8) in Doucett et al. (1996) was similar to that in the Howe Sound, British Columbia (-28.3) (Romanuk and Levings 2005), which is in the Salish Sea, close to the Comox Estuary. As terrestrial invertebrates tend to reflect their terrestrial plant food sources (Romanuk and Levings 2005), the similarity in terrestrial plant signatures between the two areas indicated that the signatures of terrestrial invertebrates would also be similar. Doucett et al.'s (1996) study was also close to our study in latitude ( $46^{\circ}52.7'\text{N}$ ), which is important because carbon isotope discrimination in plants can vary on a latitudinal gradient (Korner et al. 1991). As lipids can be depleted in  $\delta^{13}\text{C}$  relative to other tissues, and lipid concentrations can vary largely between individuals and samples, juvenile Chum salmon were lipid corrected using the following equation from Post et al. (2007):

$$\delta^{13}\text{C}_{\text{corrected}} = -3.32 + 0.99 \times \text{C:N by mass}$$

We assume a value of 0.4  $\delta^{13}\text{C}$  and 3.4 for  $\delta^{15}\text{N}$  as trophic enrichment factors for juvenile Chum salmon for each prey source to correct. These values are widely robust (Post 2002), and have been used for salmonids in other studies (eg. Hertz et al. 2016). A standard deviation of 2 for trophic enrichment factors of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was assumed. Averages and standard deviations of isotope signatures of eelgrass, epiphytes, epifaunal prey invertebrates, freshwater invertebrates, terrestrial invertebrates, zooplankton, insects, and lipid-corrected Chum salmon were calculated (Table S5). The isotopic signatures of

juvenile Chum salmon and their potential diet sources, eelgrass epifaunal invertebrates, zooplankton, and terrestrial insects, were plotted. A MixSIAR Bayesian mixing model (Stock and Semmes 2013) was used to estimate probability distributions of each diet source towards juvenile Chum salmon (Table S6). The analysis was performed using uninformative priors, then informative priors generated from our gut content analysis (2.8% for insects, 79.0% for eelgrass invertebrates, and 18.2% for zooplankton) (Table S6). All statistical analyses were conducted using R 3.2.1 (Core Team 2015), using the vegan package (Oksanen et al. 2009).

## RESULTS

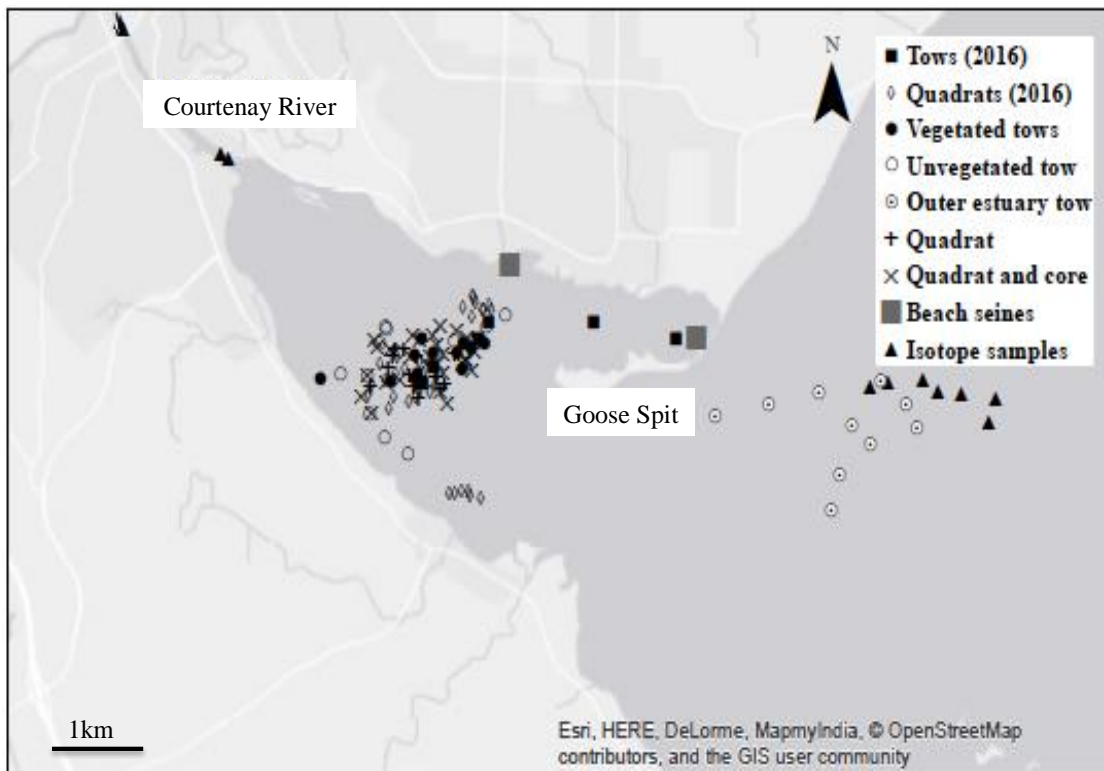


Figure 1. Map of 2015/2016 sampling sites in the Comox Estuary, British Columbia, Canada.

### Prey availability – Epifaunal eelgrass invertebrates

Eelgrass density in the Comox Estuary ranged widely, from 16-416 shoots/m<sup>2</sup> (Table 1). Eelgrass blades hosted a total of 9 epifaunal prey invertebrate groups, including polychaete worms, and crustaceans such as harpacticoid copepods, mites, corophid amphipods, tanaids, isopods, cumaceans, gammarid amphipods, and other senticaud amphipods (Table 2). While epifaunal prey invertebrate counts were variable, harpacticoid copepods were the dominant invertebrate group across all samples (Table 2).

Many eelgrass metrics, including eelgrass shoot density in the entire 0.5m<sup>2</sup> quadrat, average eelgrass shoot length, eelgrass biomass, and eelgrass surface area, were significantly correlated with eelgrass shoot density (Pearson's  $r > 0.55$ ) (Table 3). Out of the correlated variables, only eelgrass shoot density was used in the negative binomial model to avoid multicollinearity. The final model of epifaunal prey invertebrate abundance included eelgrass shoot density, epiphyte biomass (g)/shoot, and year (Table 4). There was no significant spatial autocorrelation in epifaunal prey invertebrate abundance across eelgrass quadrats taken  $\geq 50$ m apart (Moran's  $I$ :  $z = 0.940$ ,  $p = 0.347$ ).

A likelihood ratio test indicated the negative binomial model explained significantly more deviance than a Poisson model and was thus more appropriate ( $\chi^2_5 = 30780.5$ ,  $p < 0.0001$ ). When epiphyte biomass was held at its mean (52.86mg), epifaunal prey invertebrate abundance increased significantly with a coefficient of 0.1359 on the log

Table 1. Eelgrass variables in low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May, 2015-2016.

Measurement	Low (0-49 shoots/m <sup>2</sup> )		Medium (50-99 shoots/m <sup>2</sup> )		High (100+ shoots/m <sup>2</sup> )	
	2015	2016	2015	2016	2015	2016
	Average(sd)	Average(sd)	Average(sd)	Average(sd)	Average(sd)	Average(sd)
Eelgrass shoots/0.0625m <sup>2</sup>	2.2(0.8)	2(0.6)	5.3(0.9)	4.8(0.8)	12.7(5.5)	11.2(3.1)
Eelgrass shoots/m <sup>2</sup>	36.0(13.3)	32(10.7)	86.0(14.6)	76.8(12.6)	203.3(88.6)	180.3(50.8)
Average shoot length (cm)	42.7(16.0)	53.1(11.1)	62.5(20.1)	75.5(16.4)	72.9(19.0)	75.9(14.0)
Average shoot width (cm)	0.5(0.1)	0.5(0.1)	0.5(0.1)	0.5(0.1)	0.6(0.1)	0.5(0.1)
Blades per shoot	NA	4.2(0.6)	NA	4.5(0.6)	NA	4.9(0.8)
Shoot biomass (g)	0.5(0.4)	0.8(0.4)	2.6(4.5)	1.9(1.2)	5.8(6.5)	4.4(1.73)
Epiphyte biomass (mg)/shoot	41.4(49.7)	55.1(101.4)	104.3(81.2)	68.1(87.9)	30.7(39.4)	4.4(1.73)
Surface area (cm <sup>3</sup> /0.0625m <sup>2</sup> )	482.9(308.6)*	413.0(228.0)	1907.0(973.3)*	1862.5(944.0)	4669.8(3508.4*)	4955.3(2928.4)

\* Calculated using average blades per shoot from low, medium, and high shoot density categories in 2016

Table 2. Average and standard deviation (sd) of eelgrass prey invertebrate counts in low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015-2016. Averages are rounded to the nearest whole number.

Taxon	Low (0-49 shoots/m <sup>2</sup> )		Medium (50-99 shoots/m <sup>2</sup> )		High (100+ shoots/m <sup>2</sup> )	
	2015	2016	2015	2016	2015	2016
	Average(sd)	Average(sd)	Average(sd)	Average(sd)	Average(sd)	Average(sd)
Annelida						
Small polychaete worms (<5cm)	1(2.2)	1(0.5)	3(4.1)	1(2.4)	6(8.8)	3(5.2)
Crustacea						
Harpacticoid copepods	183(287.8)	144(90.8)	297(306.5)	472(577.4)	388(558.4)	1179(1196.2)
Mites	6(13.0)	0(0.3)	5(8.5)	0(0.3)	16(33.9)	3(3.8)
Amphipoda						
Malacostraca						
Corophid amphipods	2(3.0)	0(0.3)	9(16.0)	1(1.9)	12(12.9)	7(4.1)
Tanaids	0(0)	0(0)	0(0)	0(0.3)	0(0)	1(1.8)
Small isopod (<1cm)	0(0)	0(0)	0(0)	0(0.3)	0(0.3)	1(2.1)
Large isopod (>1cm)	0(0.4)	0(0)	1(1.4)	0(0.4)	2(1.5)	1(2.0)
Cumaceans	0(0.3)	0(0.4)	1(2.8)	1(3.8)	2(2.8)	1(2.1)
Senticaudata						
Gammarid amphipods	7(9.4)	2(3.0)	34(43.0)	5(6.0)	70(88.4)	7(11.62)
Other senticaud amphipods	0(0.5)	0(0)	1(2.8)	1(1.0)	1(3.0)	1(1.5)
Other amphipods	0(0)	0(0)	0(0)	0(0)	0(1.8)	0(0.3)

Table 3. Pearson correlations between variables of eelgrass collected from the Comox Estuary, British Columbia, May 2015-2016. Significant correlations (Pearson's  $r > 0.55$ ) are in bold.

	Shoots/0.125m <sup>2</sup>	Average shoot length (cm)	Average shoot width (cm)	Eelgrass biomass (g)	Epiphyte biomass (g)/shoot	Average blades/shoot	Surface area (cm <sup>3</sup> )
Shoots/0.0625m <sup>2</sup>	<b>0.67</b>	<b>0.58</b>	0.09	<b>0.67</b>	0.16	0.50	<b>0.82</b>
Shoots/0.125m <sup>2</sup>		0.53	0.15	0.53	0.10	0.45	<b>0.66</b>
Average shoot length (cm)			0.33	<b>0.67</b>	0.13	0.45	<b>0.68</b>
Average shoot width (cm)				-0.03	-0.05	-0.04	0.45
Eelgrass biomass (g)					-0.13	0.35	<b>0.57</b>
Epiphyte biomass (g)/shoot						-0.10	-0.16
Average blades/shoot							<b>0.51</b>

scale with every standard deviation increase in eelgrass shoot density (Table 4, Figure 2). Year was a significant predictor of epifaunal invertebrate abundance ( $\chi^2_{71,72} = 6.254$ ,  $p = 0.0124$ ). Epifaunal invertebrate abundance was also positively influenced by epiphyte biomass (Table 4). A potential outlier did not affect results (Table S2, Figure S2), and when all possible combinations of the additional eelgrass covariates were modeled, only shoot density was consistently in all models with a delta AIC < 2 (Table S3). In fact, models without shoot density had delta AIC values > 10, indicating low support for these models compared to ones including shoot density (Burnham and Anderson 2003), and suggesting shoot density was the most important predictor of the three covariates. The effect sizes of all other covariates were also small and their inclusion in the model did not influence the effect sizes of other variables, meaning the inclusion of all covariates would not likely influence the results.

Table 4. Summary of negative binomial model of epifaunal prey invertebrate abundance in eelgrass quadrats from the Comox Estuary, British Columbia, May 2015-2016.

	Estimate	SE	Z	P-value
Intercept	4.786407	0.228158	20.978	<0.0001
Year*	0.702614	0.265388	2.647	0.0081
Shoots collected*	0.135946	0.024616	5.523	<0.0001
Epiphyte biomass (mg/shoot)*	0.006451	0.001743	3.700	0.0002

Null deviance: 126.240 on 74df, residual deviance: 90.861 on 71df, 2 x log-likelihood: -1038.205 \*Data are centered

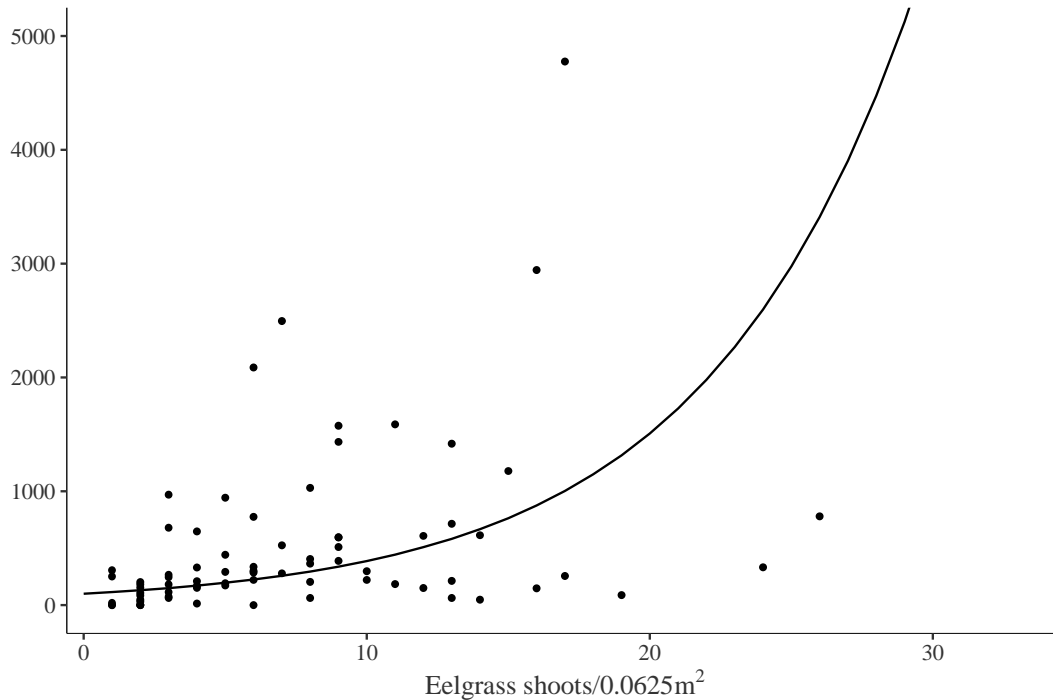


Figure 2. Predicted values of negative binomial model of epifaunal prey invertebrate abundance as a function of shoot density in 0.0625m<sup>2</sup> eelgrass quadrats collected in May 2015 and 2016 in the Comox Estuary, British Columbia. Year is held at its centered value, and epiphyte biomass at its mean.

There was no significant autocorrelation in epifaunal prey invertebrate communities at a distance of 50m based on a Mantel test with 1000 permutations ( $p = 0.171$ ). The CAP of epifaunal prey invertebrate abundance was significant based on an ANOVA-like permutation test with 999 permutations ( $F_{3,64} = 4.687$ ,  $p = 0.001$ ), explaining 17.6% of the variation in the epifaunal prey invertebrate community. The explanatory variables were standardized so that the length of the arrows on the CAP indicated the relative influence of each variable on prey invertebrate community composition (Figure 3). Eelgrass shoot density had a significant effect on epifaunal prey invertebrate abundance ( $F_{1,66} = 6.128$ ,  $p = 0.001$ ) explaining 7.7% of the variation in epifaunal prey invertebrate communities. The effects of epiphyte biomass and year were also significant ( $F_{1,66} =$

1.821,  $p = 0.001$  and  $F_{1,66} = 5.208$ ,  $p = 0.001$ , respectively), explaining 6.6% and 2.2% respectively (Figure 3). The first axis of the CAP explained 68.3% of the total constrained variation in epifaunal prey invertebrate composition, and the second axis explained 18.9% (Figure 3). High-density eelgrass shoot quadrats separated from low and medium eelgrass shoot density quadrats along the first axis (Figure 3). All prey invertebrates groups, except for tanaids, were positively associated with eelgrass shoot density and epiphyte biomass (Figure 3). The score for tanaids on the first axis is near the centroid for 2016, potentially indicating a higher abundance of tanaids in 2016 compared to 2015 (Figure 3). Harpacticoid copepods displayed strong negative correlations with the second constrained axis, meaning the second axis largely separated quadrats with an abundance of harpacticoid copepods from quadrats without (Figure 3).

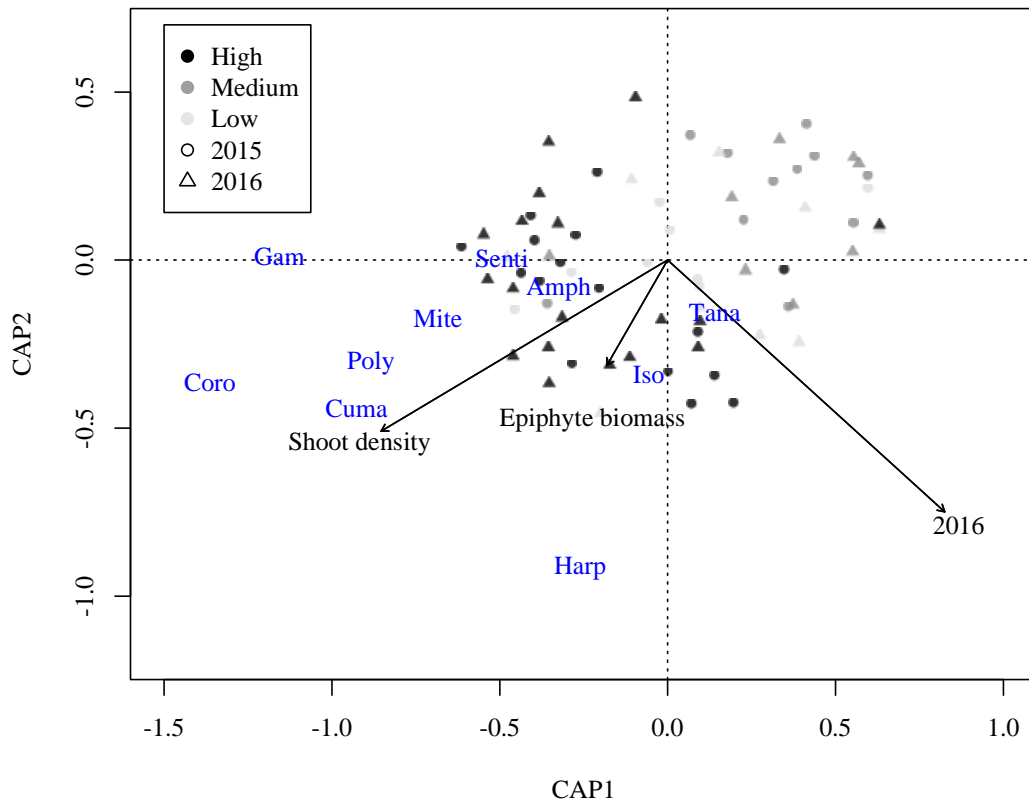


Figure 3. Constrained analysis of principal coordinates (CAP) of epifaunal prey invertebrate communities across low, medium, and high-density eelgrass collected from 0.0625m<sup>2</sup> quadrats in May 2015-2016 from the Comox Estuary, British Columbia. Shoot density, epiphyte biomass, and year are explanatory variables, with length of the arrows indicating the strength of each predictor. Each point on the graph represents the epifaunal prey invertebrates found in a single quadrat, with density categories coded as colours and year as different shapes. Species are abbreviated for display purposes as follows: Gam = gammarid amphipods, Senti = other senticaud amphipods, Amph = other amphipods, Tana = tanaids, Coro = corophid amphipods, Poly = polychaete worms, Iso = isopods, Mite = mites, Harp = harpacticoid copepods, Cuma = cumaceans. Shoot density is shoots/0.0625m<sup>2</sup> quadrat.

#### Prey availability – Infaunal eelgrass invertebrates

Seven infaunal prey invertebrate groups were found in the infaunal eelgrass prey invertebrate samples across the entire range of eelgrass shoot density (Table 5).

Harpacticoid copepods and small polychaete worms had the highest counts and

frequencies of occurrence across samples taken from all levels of shoot density (Table 5). There was no significant spatial autocorrelation in infaunal prey invertebrate abundance in infaunal eelgrass prey invertebrate samples taken  $\geq 50\text{m}$  apart (Moran's  $I$ :  $z = -0.778$ ,  $p = 0.430$ ).

A likelihood ratio test indicated that the negative binomial model provided a better fit to the data than a Poisson model, explaining significantly more deviance ( $\chi^2_5 = 31530.4$ ,  $p < 0.0001$ ). Eelgrass shoot density, sediment depth, and percent fine sediment were not significant predictors of infaunal prey invertebrate abundance (all  $p < 0.05$ ) (Table 6).

There was no significant autocorrelation in infaunal prey invertebrate communities at a distance of 50m based on a Mantel test with 1000 permutations ( $p = 0.220$ ). The CAP model was significant based on an ANOVA-like permutation test with 999 permutations ( $F_{3,26} = 1.685$ ,  $p = 0.013$ ) (Figure 4). The CAP explained 17.5% of the variation in infaunal prey invertebrate composition. Eelgrass shoot density, however, had no significant effect on infaunal prey invertebrate communities ( $F_{1,28} = 1.492$ ,  $p = 0.112$ ), nor did percent fine sediment ( $F_{1,28} = 1.096$ ,  $p = 0.350$ ). Sediment depth was the only significant predictor variable of infaunal prey invertebrate communities, ( $F_{1,28} = 2.209$ ,  $p = 0.018$ ), explaining 7.6% of the constrained variation. The first axis explained 59.1% of the constrained variation of the CAP. Cumaceans, gammarid amphipods, and sediment depth all had strong negative correlations with this axis. The second axis explained 24.8% of the constrained variation and correlated positively with

Table 5. Average and standard deviation (sd) of shoots/m<sup>2</sup> and average, standard deviation, and frequency of occurrence (FO) of infaunal prey invertebrate counts in 10cm deep, 8cm diameter sediment cores from quadrats in low (n=11), medium (n=5), and high (n=13) eelgrass shoot density categories in the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.

Taxon/Measure	Low (0-49 shoots/m <sup>2</sup> )		Medium (50-99 shoots/m <sup>2</sup> )		High (100+ shoots/m <sup>2</sup> )	
	Average (sd)	FO	Average (sd)	FO	Average (sd)	FO
Average shoots/m <sup>2</sup>	32(16)		86(14.3)		233(99.5)	
Annelida						
Small polychaete worms (<5cm)	17(12.8)	81.8	27(29.4)	100.0	30(54.9)	83.3
Other	2(2.8)	45.4	4(3.2)	83.3	2(2.3)	50.0
Crustacea						
Harpacticoid copepods	42(96.4)	63.6	7(4.6)	100.0	29(54.4)	66.7
Amphipoda						
Malacostraca						
Corophid amphipods	3(3.4)	63.6	6(6.6)	66.7	1(1.8)	41.6
Tanaids	9(19.9)	36.3	3(5.8)	16.6	2(7.2)	8.3
Cumaceans	4(3.7)	63.6	2(2.4)	83.3	8(10.2)	50.0
Senticaudata						
Gammarid amphipods	3(5.1)	45.4	5(9.2)	66.7	5(7)	50.0

Table 6. Summary of negative binomial model of infaunal prey invertebrate abundance as a function of shoot density, sediment depth, and percent fine sediment in the Comox Estuary, British Columbia, May 2015.

	Estimate	SE	Z	P-value
Intercept	1.47489	0.18147	22.857	<0.0001
Shoot density	0.01228	0.02519	0.484	0.628
Sediment depth	0.21903	0.15218	1.439	0.150
Fine sediment (%)	0.51677	0.777922	0.663	0.507

Log likelihood: -288.8, Null deviance 35.5 on 27df, Residual deviance 31.5 on 24df

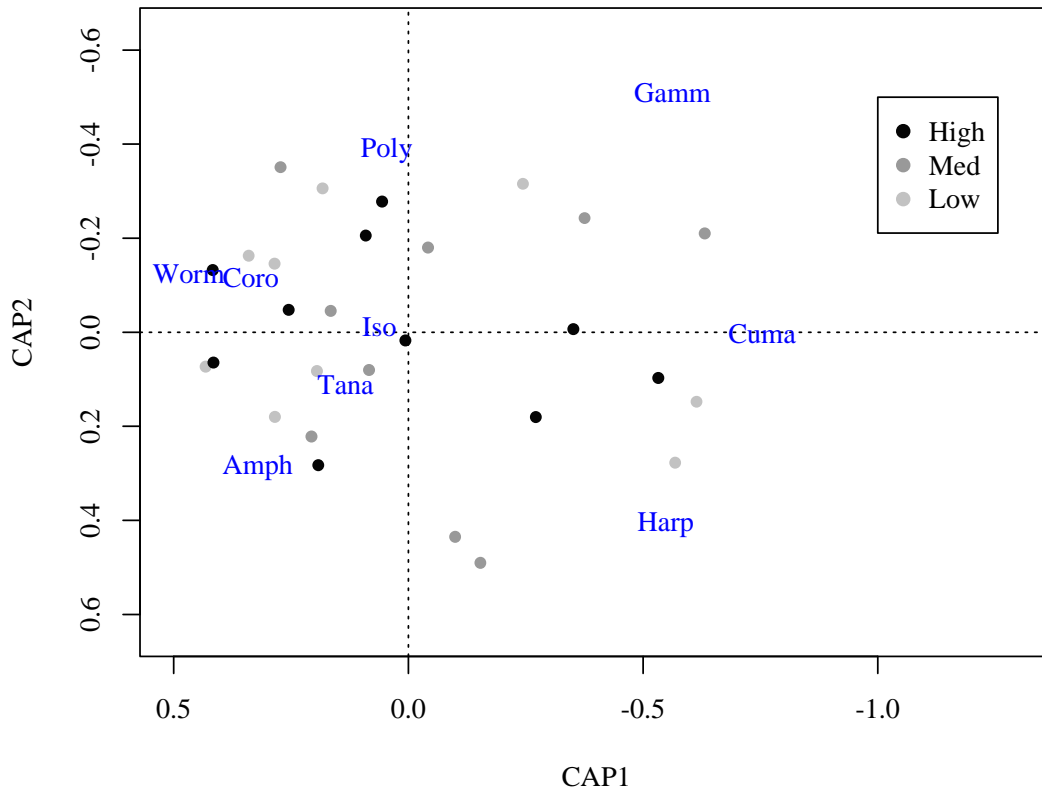


Figure 4. Constrained analysis of principal coordinates (CAP) of infaunal prey invertebrate communities in sediment cores from low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015, with eelgrass shoot density, sediment depth, and percent of fine sediments as explanatory variables. Eelgrass density categories are coded as different colours, and each point on the graph represents the infaunal invertebrates found in a single sediment core. Species are abbreviated as follows: Gam = gammarid amphipods, Amph = other amphipods, Tana = tanaids, Poly = polychaete worms, Iso = isopods, Harp = harpacticoid copepods, Cuma = cumaceans, worm = other benthic annelids. Shoot density is shoots/0.0625m<sup>2</sup> in the quadrat sediment cores were collected from, fine sediment is the dry weight of sediment <125 µm/ total dry weight of sediment.

harpacticoid copepods and eelgrass shoot density. In contrast, sediment depth, polychaete worms, and gammarid amphipods had strong negative correlations along this axis. Overall, the infaunal prey invertebrate communities across samples from areas of all eelgrass shoot

densities were scattered throughout the biplot, meaning infaunal prey invertebrate communities were similar across eelgrass shoot density categories.

#### Prey availability – Zooplankton

Juvenile fish, insects, polychaete worms, sea snails, as well as twelve types of crustaceans were found in the zooplankton samples from 2015 (Table 7). Many taxa, including juvenile fish, harpacticoid copepods, calanoid copepods, barnacle cyprids, crab and shrimp zoea, isopods, cladocerans, sea snails, and polychaete worms seemed to be more abundant in vegetated areas of the estuary than other locations; however, crustacean nauplii numerically dominated the zooplankton, and data were variable (Table 7). Eelgrass associated invertebrates, such as polychaete worms, and crustaceans such as harpacticoid copepods, mites, corophid amphipods, tanaids, isopods, cumaceans, gammarid amphipods, and other senticaud amphipods, were not abundant in the zooplankton during the day in 2015, nor during the day or evening in 2016 (Table 7, Table 8).

The CAP of zooplankton communities was not significant overall ( $F_{6,30} = 1.419$ ,  $p = 0.729$ ) (Figure 5), indicating that salinity, temperature, volume of seawater sampled, bottom depth, and the presence of eelgrass in the estuary were not significant predictors of zooplankton communities.

### Juvenile salmon diet - Stomach contents

We examined 166 juvenile Chum salmon and 37 juvenile Chinook salmon individuals for diet contents. A variety of prey items were observed in juvenile Chum salmon diets, including zooplankton-associated species such as shrimp zoea, decapod shrimp, crab zoea, crab megalopae, euphasid larvae, ostracods, cladocerans, nauplii, cyprids,

Table 7. Temperature, salinity, and bottom depth of zooplankton tows, and average, standard deviation (sd) and total density/m<sup>3</sup> (Total) of zooplankton caught in inner-estuary tows over eelgrass and unvegetated sediment, and tows outside the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.

Measure/taxon	Unvegetated estuary		Vegetated estuary		Outer estuary	
	Average(sd)	Total	Average(sd)	Total	Average(sd)	Total
Temperature °C	15.0(1.8)		14.3(0.7)		13.3(.3)	
Salinity (ppt)	29.5(0.6)		29.6(0.9)		30.4(0.2)	
Depth (m)	2.1(0.9)		2.3(0.6)		>5	
Chordata						
Juvenile fish	0(0.5)	3	6(8.8)	73	0(0.6)	3
Annelida						
Small Polychaete worms (<5cm)	3(7.1)	41	13(28.8)	155	8(9.0)	93
Mollusca						
Sea snails	0(0)	0	1	8	0(0.3)	2
Crustacea						
Harpacticoid copepods	4(6.4)	45	27(56.7)	320	2(4.2)	24
Calanoid copepods	238(241.9)	2853	1876(1445.7)	22512	720(540.9)	8636
Barnacle cyprids	51(55.7)	613	62(111.7)	746	56(35.3)	668
Shrimp zoea	8(17.9)	93	26(45.2)	315	14(14.7)	172
Crab zoea	5(5.8)	57	22(62.5)	270	7(10.4)	90
Crab megalops	0(0)	0	0(0)	0	0(0.4)	2
Euphasiid zoea	1(1)	9	11(27.4)	130	7(6.5)	81
Cladocerans	292(396.0)	3505	1534(2564.8)	18413	281(422.3)	3372
Amphipoda						
Malacostraca						
Small isopod (<1cm)	0	0	1(2.7)	15	0(0)	0
Hyperid amphipods	0(1.4)	5	0(0)	0	1(2.7)	17
Senticaudata						
Gammarid amphipods	0(0)	1	0(0)	0	0(0)	0
Insecta						
Dipterans	0(0)	1	0(1)	1	0(0)	0

\*Salinity, temperature taken at 1m depth

Table 8. Species occurrences in daytime (15:00-18:00) and night-time (21:00-23:00) invertebrate sweeps over eelgrass in the Comox Estuary, British Columbia, May 2016.

Taxa	Day Tows (6)	Night Tows (6)
Annelida		
Small Polychaete worms (<5cm)	5	5
Mollusca		
Snails	2	2
Crustacea		
Crustacean nauplii	6	6
Harpacticoid copepods	5	1
Calanoid copepods	6	6
Cyclopoid copepods	1	0
Barnacle cyprids	6	4
Shrimp zoea	6	6
Decapod shrimp	3	1
Ostracods	6	6
Crab zoea	6	6
Crab megalopae	3	5
Cladocerans	1	2
Amphipoda		
Malacostraca		
Tanaids	0	1
Small isopod (<1cm)	1	0
Cumaceans	1	3
Senticaudata		
Gammarid amphipods	1	3
Hyperid amphipods	5	5
Insecta		
Dipterans	0	2
Vertebrata		
Juvenile fish	0	2

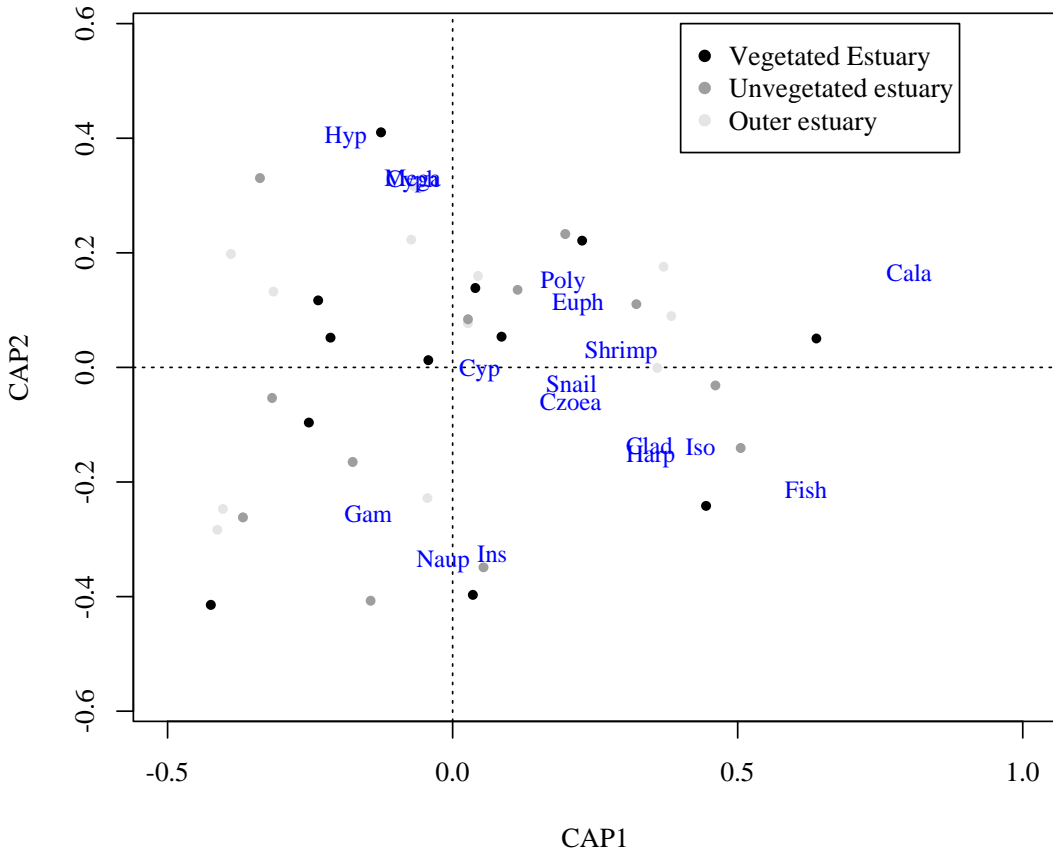


Figure 5. Constrained analysis of principal coordinates (CAP) of zooplankton communities from tows over eelgrass in vegetated and unvegetated estuary, and outer-estuary in Comox British Columbia, May 2015. Tow type is the explanatory variable, and is coded as different colours, with each point on the graph representing the invertebrates found in a single tow. Species are abbreviated for display purposes as follows: Amph = other amphipods, Poly = polychaete worms, Iso = isopods, Harp = harpacticoid copepods, Ins = terrestrial insects, Naup = crustacean nauplii, Hyp = hyperid amphipods, Cyp = barnacle cyprids, Mega = crab megalopae, Snail = sea snails, C zoea = crab zoea, Shrimp = shrimp zoea, Fish = larval fish, Cala = calanoid copepods, Clad = cladocerans, Euph = euphasid zoea, Cyclo = cyclopoid copepod, Cyph = cyphanaules larvae.

hyperid amphipods, and gammarid amphipods, terrestrial species including spiders and dipterans; and benthically associated species, such as gammarid amphipods, harpacticoid copepods, polychaete worms, cumaceans, and snails (Table 9). Similarly, a combination of terrestrial and aquatic invertebrates were found in Chinook salmon stomachs, including zooplankton-associated species, such as shrimp zoea, decapod shrimp, crab zoea, crab

megalopae, euphasid larvae, ostracods, cladocerans, crustacean nauplii, cyprids, hyperid amphipods; terrestrial species such as ants; and benthic species such as gammarid amphipods, polychaete worms, harpacticoid copepods, and tanaids (Table 10).

The species accumulation curve reached an asymptote at 166 juvenile Chum salmon in the Comox Estuary (minimum derivative = 0.0169), indicating the diets of juvenile Chum salmon were adequately sampled (Figure 6). The species accumulation curve did not reach an asymptote for the 37 juvenile Chinook salmon caught in the Comox Estuary, potentially indicating that the sampling effort for juvenile Chinook salmon was not sufficient to fully describe their diets (Figure 6). However, the proximity of the minimum derivative for juvenile Chinook salmon diets (0.0786) to the cut off value of 0.05 (Figure 6), likely means the number of Chinook salmon sampled was adequate to give a general idea of juvenile Chinook salmon diets in the Comox Estuary.

Harpacticoid copepods dominated the invertebrate counts in both juvenile Chum salmon and juvenile Chinook salmon stomachs (Figure 7, Table 9, Table 10). Polychaete worms and cladocerans were the next most important groups for juvenile Chum salmon, while decapod shrimp and crab megalopae were the next most important for juvenile Chinook salmon (Figure 7). Benthic species made up 79.0% and 47.8% of Chum salmon and Chinook salmon diets, respectively (Figure 7). Planktonic species made up 18.2% and 30.0% of juvenile Chum salmon and juvenile Chinook salmon diets respectively, followed by terrestrial insects, which made up 2.8% and 20.2%, respectively. When data were

Table 9. Salmon metrics, average, standard deviation (sd), and frequency of occurrence (FO) of invertebrate counts in juvenile Chum salmon diets by tow number from the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.

Taxon/measure	Tow 1 (n=40)		Tow 2 (n=18)		Tow 3 (n=6)		Tow 4 (n=75)		Tow 5 (n=27)	
	Average	FO	Average	FO	Average	FO	Average	FO	Average	FO
Length (mm)	56(4.6)		44(4.9)		65(10.0)		50(5.3)		52(5.2)	
Relative weight (%)	116.3(10.9)		112.5(21.6)		97.8(7.4)		89.9(8.5)		93.5(16.6)	
Number of diets in % digested categories 1,2,3	4,1,35		0,3,15		0,0,6		1,1,73		0,0,27	
Chordata										
Juvenile fish	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	0(0.1)	1.3	0(0.0)	0.0
Annelida										
Small Polychaete worms (<5cm)	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	18(37.9)	38.7	17(43.4)	40.7
Gastropoda										
Snails	1(2.6)	20.0	0(0.0)	0.0	0(0.0)	0.0	0(0.3)	8.0	0(0.2)	3.7
Crustacea										
Harpacticoid copepods	836(296.1)	97.5	144(121.6)	94.4	95(99.8)	100.0	26(47.3)	84.0	179(296.8)	92.6
Calanoid copepods	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	0(0.3)	8.0	0(0.0)	0.0
Shrimp zoea	4(16.1)	37.5	1(2.8)	50.0	1(1.5)	50.0	2(6.4)	33.3	3(10.4)	18.5
Decapod shrimp	2(9.5)	10.0	1(1.0)	33.3	2(2.6)	33.3	0(0.0)	0.0	0(0.2)	3.7
Crab zoea	0(1.8)	10.0	0(0.5)	5.5	1(3.3)	16.6	0(0.0)	10.7	0(0.0)	7.4
Crab megalops	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	0(0.2)	2.7	0(0.0)	0.0
Euphasid larvae	4(17.1)	22.5	0(0.0)	0.0	0(0.0)	0.0	7(17.5)	64.0	10(33.6)	33.3
Ostracods	9(1.2)	7.5	2(2.6)	27.8	0(0.0)	0.0	0(0.6)	2.6	1(2.4)	7.4
Cladocerans	17(54.9)	27.5	22(50.6)	50.0	0(0.0)	0.0	0(0.4)	4.0	1(2.3)	14.8
Crustacean nauplii	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	0(0.1)	1.3	0(0.0)	0.0
Barnacle cyprids	5(8.4)	52.5	3(3.9)	55.6	0(0.0)	0.0	1(1.4)	36.0	1(1.9)	44.4
Amphipoda										
Malacostraca										
Corophid amphipods	0(0.0)	0.0	0(0.4)	16.7	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0
Cumaceans	0(0.2)	5.0	0(1.0)	11.1	0(0.0)	0.0	0(1.2)	14.7	0(0.4)	3.7
Senticaudata										
Gammarid amphipods	0(1.9)	0.0	0(0.5)	5.6	0(0.0)	0.0	0(0.5)	8.0	0(0.0)	3.7
Hyperid amphipods	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	0(0.8)	30.7	0(0.0)	0.0
Other amphipods	0(1.9)	5	0(0.0)	0.0	0(0.0)	0.0	0(0.0)	0.0	0(0.4)	2.6
Insecta										
Dipterans	3(5.2)	57.5	6(3.9)	83.3	23(31.8)	100.0	0(0.8)	20.0	0(0.6)	30.0
Arachnids	0(0.0)	0.0	0(0.2)	3.5	1(0.8)	50.0	0(0.0)	0.0	0(0.0)	0.0

Table 10. Average, standard deviation (sd), and frequency of occurrence (FO) of invertebrate counts in Chinook salmon diets by tow number from the Comox Estuary, British Columbia, May 2016. Averages are rounded to the nearest whole number.

Taxon/measure	Tow 1 (n=5)		Tow 2 (n=31)	
	Average(sd)	FO	Average(sd)	FO
Length (mm)	62(17.1)		55.3(6.1)	
Relative weight (%)	90.2(3.2)		84.3(12.3)	
Number of diets in % digest categories 1,2,3	0,1,4		0,0,31	
Annelida				
Small polychaete worms (<5cm)	1(0.4)	20.0	0(0.0)	0.0
Crustacea				
Harpacticoid copepods	1(1.3)	20.0	37(92.7)	67.7
Calanoid copepods	0(0.0)	0.0	0(0.6)	22.6
Decapod shrimp	7(14.8)	20.0	2(2.9)	54.8
Crab megalops	8(9.2)	60.0	1(2.2)	12.9
Euphasid larvae	0(0.0)	0.0	0(0.2)	3.0
Crustacean nauplii	1(0.4)	20.0	0(0.2)	3.2
Amphipoda				
Malacostraca				
Tanaids	0(0.4)	20.0	1(3.0)	32.2
Hyperid amphipods	1(1)	60.0	1(0.9)	25.8
Senticaudata				
Gammarid amphipods	1(0.4)	20.0	1(1.2)	54.8
Insecta				
Dipterans	0(0.0)	0.0	0(0.2)	3.2
Ants	0(0.0)	0.0	0(0.9)	16.1

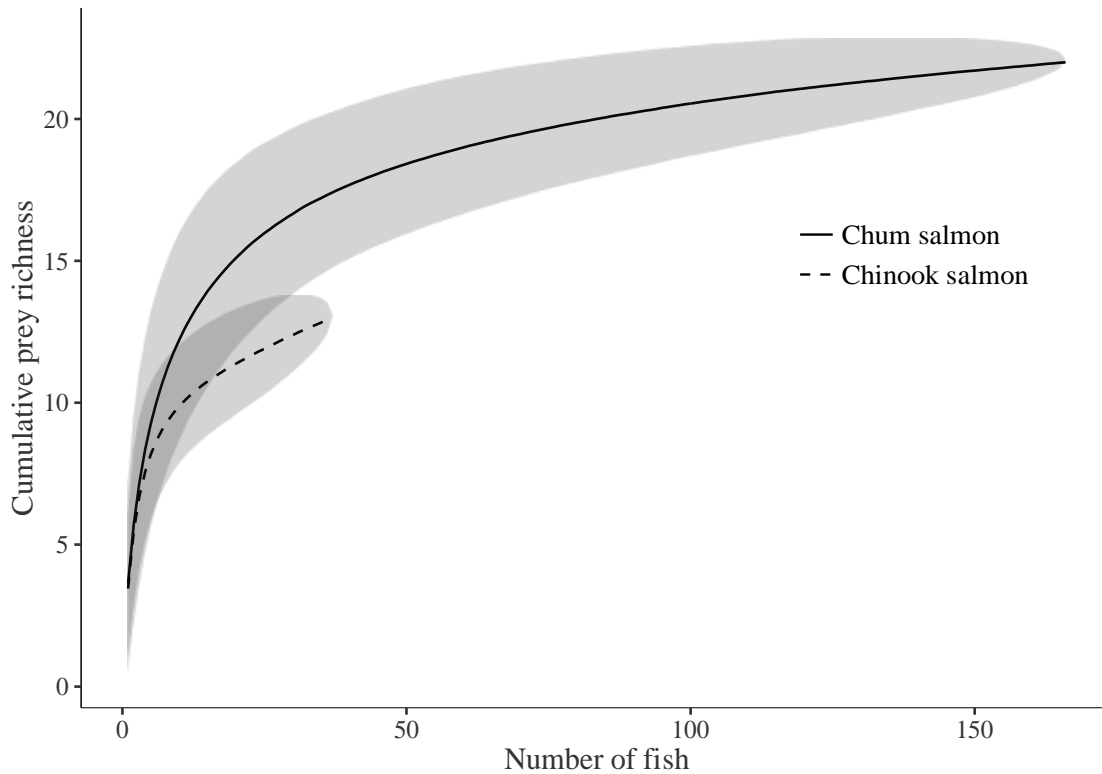


Figure 6. Prey accumulation curves for diets of juvenile Chum salmon caught in May 2015, and juvenile Chinook salmon caught in May, 2016 in the Comox Estuary, British Columbia.

analyzed by weight instead of counts for Juvenile Chum salmon, diets patterns were similar (Figure S4).

The CAP of juvenile salmon diets was significant based on an ANOVA like permutation test with 999 permutations ( $F_{5,160} = 4.962$ ,  $p = 0.001$ ) Figure 8). Salmon metrics, including juvenile salmon fork length, condition, gut fullness, and digestion score described a total of 10.1% of the variation in juvenile salmon diets when the effects of tow were removed (Figure 8). Gut fullness ( $F_{1,165} = 3.3713$ ,  $p = 0.018$ ), and species ( $F_{1,165} = 8.8616$ ,  $p = 0.001$ ) were significant predictors of juvenile salmon diets based on a permutation test

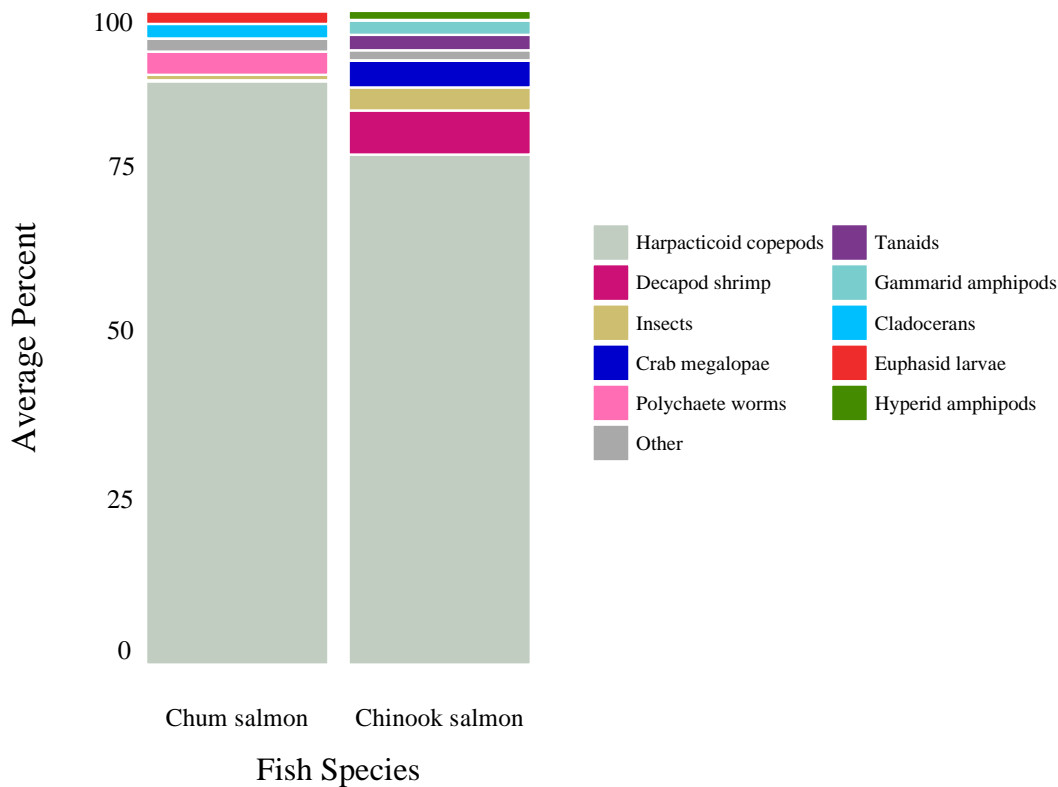


Figure 7. Average percent of invertebrate counts in diets of juvenile Chum salmon caught in 2015 and juvenile Chinook salmon caught in 2016 in the Comox Estuary, British Columbia.

with 999 permutations, but only explained 0.04% and 0.02% of the variation, respectively (Figure 8). The first axis explained 52.5% of the total constrained variation in juvenile salmon diets, largely separating juvenile Chinook salmon, which had mean positive scores with the first axis, from juvenile Chum salmon, which had mean negative scores with the first axis. The second axis explained 15.5% of the total constrained variation (Figure 8). Gut fullness was positively associated with the second axis, along with shrimp zoea and euphausids, suggesting these invertebrates made up a larger proportion of the diets when stomachs were fuller (Figure 8).

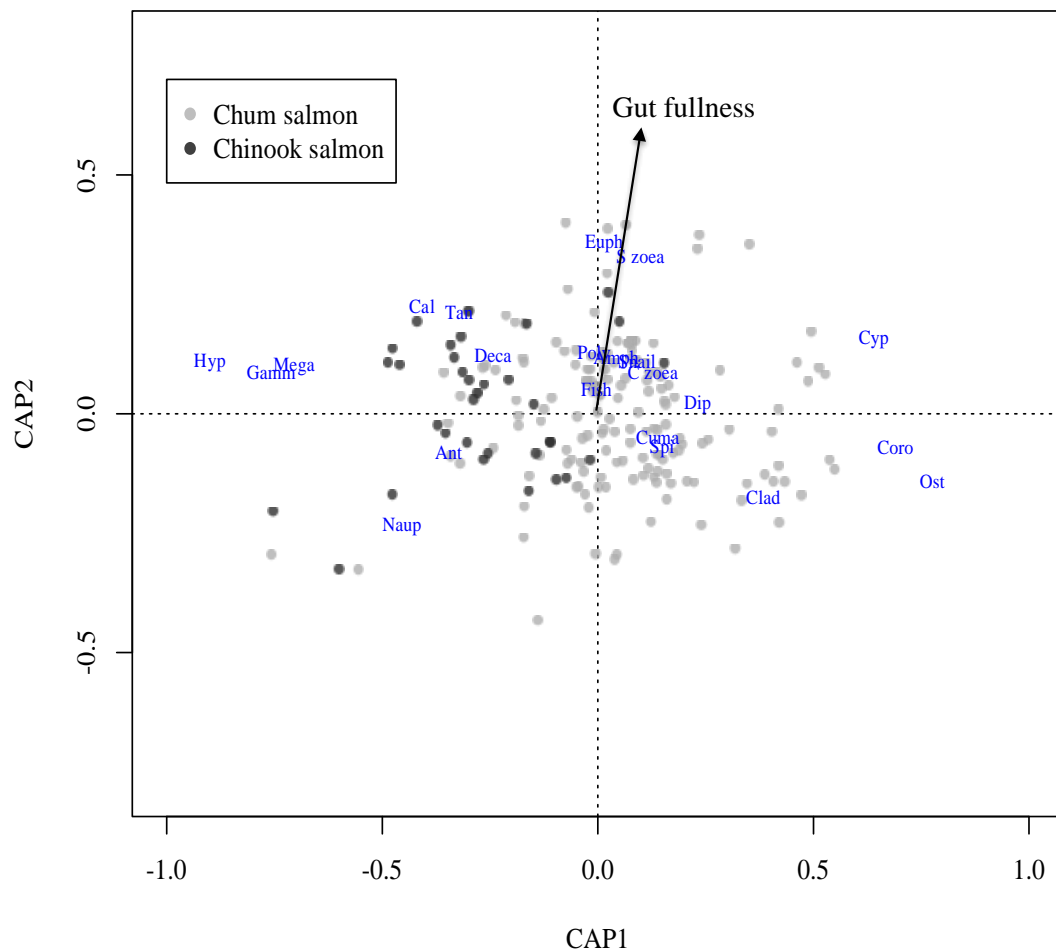


Figure 8. Constrained analysis of principal coordinates (CAP) of juvenile salmon diets in the Comox Estuary, British Columbia, May 2015. Salmon species is the explanatory variable coded as different colours, with each point on the graph representing the invertebrates found in an individual diet. Species are abbreviated for display purposes as follows: Amph = other amphipods, Poly = polychaete worms, Iso = isopods, Harp = harpacticoid copepods, Ins = terrestrial insects, Naup = crustacean nauplii, Hyp = hyperid amphipods, Cyp = barnacle cyprids, Mega = crab megalops, Snail = sea snails, C zoea = crab zoea, Shrimp = shrimp zoea, Fish = larval fish, Cala = calanoid copepods, Clad = cladocerans, Euph = euphasid zoea, cyclo = cyclopoid copepod, cyph = cyphanaules larvae, Ant = terrestrial ants, Coro = corophid amphipods, Dip = dipteran flies, Deca = decapod shrimp, Ost = ostracods, Spi = spiders, Tan = tanais.

Copepod length data met the assumption of homogeneity of variance based on a Levene's test ( $F_{2, 90}=1.891$ ,  $p=0.1368$ ). While copepods were not identified down to species, there were significant differences in average copepod length between samples as determined by

a one-way ANOVA ( $F_{3,89} = 18.87$ ,  $P < 0.0001$ ) (Figure 9). Average copepod length in juvenile Chum salmon gut contents ( $0.96 \pm 0.28 \mu\text{m}$ ), was significantly larger than that in epifaunal eelgrass samples ( $0.60 \pm 0.07 \mu\text{m}$ ), infaunal eelgrass samples ( $0.27 \pm 0.47 \mu\text{m}$ ), and zooplankton samples ( $0.7 \pm 0.22 \mu\text{m}$ ) as determined by Tukey's HSD ( $p < 0.0001$ ,  $< 0.0001$ , and  $0.008$ , respectively). Zooplankton cyclopoid copepods were significantly larger than harpacticoid copepods in infaunal samples ( $p = 0.006$ ), but not in epifaunal samples ( $p = 0.104$ ). There was no difference between harpacticoid copepod length between infaunal and epifaunal eelgrass samples ( $p = 0.358$ ) (Figure 9).

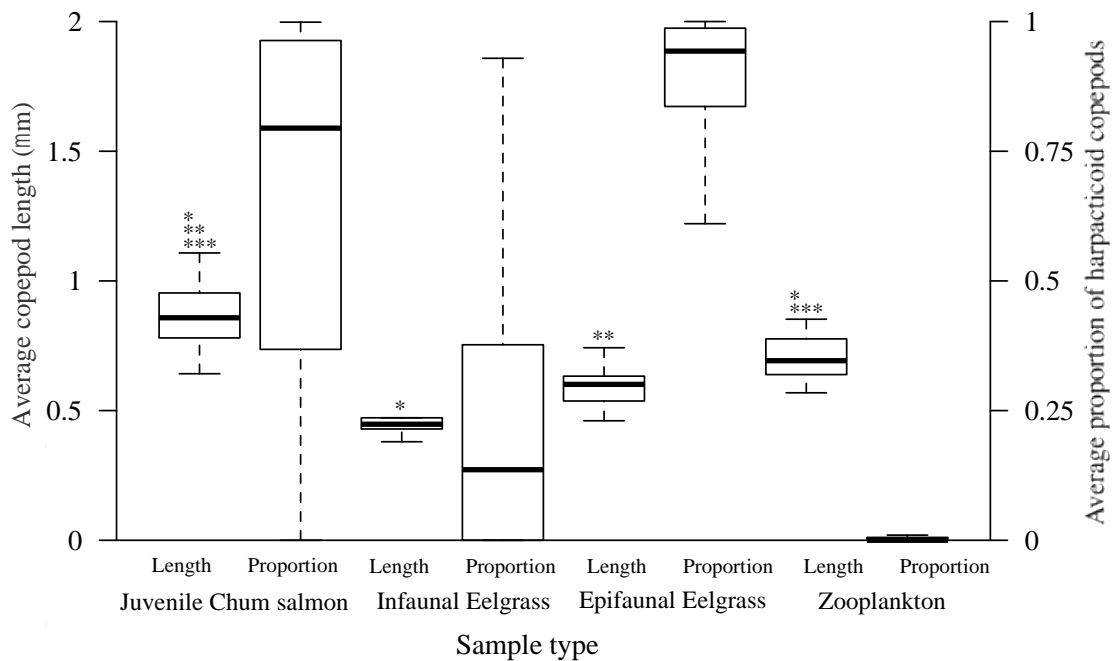


Figure 9. Average length of copepods, and average proportion of harpacticoid copepods found in juvenile Chum salmon gut contents, infaunal eelgrass invertebrate samples, epifaunal eelgrass invertebrate samples, and zooplankton samples from the Comox Estuary, British Columbia, May 2015. Significant differences in average copepod lengths indicated by asterisks.

## Juvenile salmon diet – Stable isotope analysis

Stable isotope analysis revealed that terrestrial insects were depleted in  $\delta^{13}\text{C}$  ( $-27.00 \pm \text{sd}2$ ) relative to other sources, while eelgrass was relatively enriched ( $-9.43 \pm \text{sd}0.76$ ) (Table 11, Figure 10). Stable isotope signatures of juvenile Chum salmon closely matched those of eelgrass invertebrates (Figure 10). Using uninformative priors, eelgrass invertebrates made up the highest mean percent of juvenile Chum salmon diet ( $79.8 \pm \text{sd}10.2\%$ ), with Bayesian credible intervals ranging from 54.6-95.2% (Table S5). The second highest contributor to juvenile Chum salmon diets was zooplankton ( $16.9 \pm 11.0\%$ ), followed by insects, which had the lowest estimated contribution to juvenile Chum salmon diet ( $4.2 \pm 3.4\%$ ) (Table S5). Using informative priors, eelgrass invertebrates again made up the highest mean percent of juvenile Chum salmon diet ( $82.5 \pm 8.3$ ) with Bayesian credible intervals ranging from 6.2-94.5% (Table S5). The relative importance of insects and zooplankton changed, however, with insects having a higher importance by mean proportion of diet ( $8.9 \pm 4.2\%$ ), followed by zooplankton, which had the lowest estimated contribution to juvenile Chum salmon diet ( $8.6 \pm 9.0\%$ ) (Table S5).

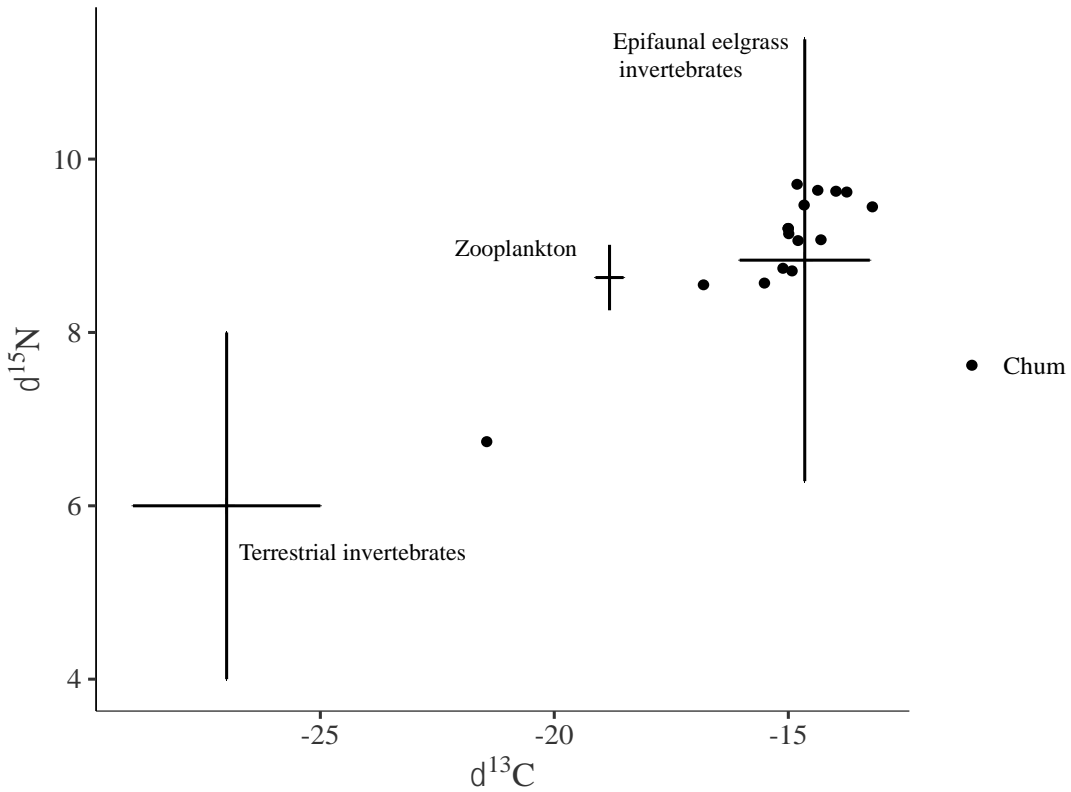


Figure 10.  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  stable isotope signatures of juvenile Chum salmon and three potential carbon sources (epifaunal eelgrass prey invertebrates, zooplankton, and terrestrial invertebrates) from the Comox Estuary, British Columbia, 2015-2016. Juvenile Chum salmon were corrected for lipids and a trophic enrichment factor of 0.4 for  $\delta^{13}\text{C}$  and 3.4 for  $\delta^{15}\text{N}$ .

Table 11. Average, standard deviation (sd), and sample size (n) of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  isotope signatures and trophic enrichment factors for juvenile Chum salmon and potential diet sources and resources in the Comox Estuary, British Columbia, May 2015.  $\delta^{13}\text{C}$  signatures of juvenile Chum salmon have been lipid corrected.

Source/Consumer	Isotope signatures				Trophic enrichment factor				n
	Mean $\delta^{13}\text{C}$	sd $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	sd $\delta^{15}\text{N}$	Mean $\delta^{13}\text{C}$	sd $\delta^{13}\text{C}$	Mean $\delta^{15}\text{N}$	sd $\delta^{15}\text{N}$	
Terrestrial insects	-27.00	2.00	6.00	2.00	0.4	1	3.4	1	4
Eelgrass prey invertebrates	-14.65	1.39	8.83	2.55	0.4	1	3.4	1	6
Eelgrass	-9.43	0.76	6.94	0.57	NA	NA	NA	NA	7
Freshwater invertebrates	-18.33	0.87	7.55	2.01	0.4	1	3.4	1	7
Zooplankton	-18.82	0.30	8.63	0.38	0.4	1	3.4	1	0
Epiphytes	-13.17	1.76	7.24	0.57	NA	NA	NA	NA	6
Juvenile Chum salmon	-14.79	1.85	12.43	0.72	NA	NA	NA	NA	6

## DISCUSSION

The overall goal of this study was to quantify the importance of eelgrass habitat in creating juvenile salmon foraging opportunities. Our first objective was to understand how infaunal and epifaunal eelgrass prey invertebrate communities differed in abundance and structure with variation in eelgrass density and form. Our hypothesis that eelgrass increased the abundance of epifaunal and infaunal prey invertebrates was supported by epifaunal samples, but not infaunal samples. We hypothesized that these prey invertebrate communities would also vary with eelgrass density, but found that in the Comox Estuary, eelgrass prey invertebrate composition remains largely unchanged across eelgrass density. Our second objective was to quantify the importance of infaunal and epifaunal eelgrass invertebrates in juvenile salmon diets. We hypothesized that eelgrass would host an abundance of prey items targeted by juvenile salmon, and that the signatures of juvenile salmon would reflect those of eelgrass epifaunal invertebrates.

Our study found that eelgrass blades and associated sediments hosted a variety of prey invertebrates consumed by juvenile salmon. The most common taxon in juvenile salmon diets was harpacticoid copepods, which we only found in high abundances in eelgrass habitat. Stable isotope analysis complemented these findings by revealing that the diets of juvenile Chum salmon were comprised mainly of benthic invertebrates. As the abundance of harpacticoid copepods and other benthic prey invertebrates increased with higher eelgrass shoot densities, we conclude that habitat features like eelgrass increase prey abundance in near-shore environments. As such, these features are likely

key to salmon populations, especially given the importance of growth and survival in the early marine stage (Beamish 2010). We believe efforts should be made to conserve these crucial habitats, and that further research should be conducted with a focus on the influence eelgrass has on overall habitat productivity, and the that effect estuarine health has on juvenile salmon survival.

#### Prey availability – Epifaunal eelgrass invertebrates

Overall, eelgrass habitat in the Comox Estuary hosted a variety of potential prey for juvenile salmon, including harpacticoid copepods, polychaete worms, amphipods, isopods, tanaids, and cumaceans. The invertebrate species found in the Comox Estuary were similar to those found in Knight et al.'s (2015) study in Tsawwassen, BC, also in the Salish Sea. Consistent with our hypothesis, and supported by a vast body of literature (Orth et al. 1984, Bostrom et al. 2006, Hosack et al. 2006, Moller et al. 2014), the abundance of these epifaunal prey invertebrates in the Comox Estuary increased with eelgrass shoot density. The negative binomial model we used also indicated a positive influence of epiphyte load on the total abundance of epifaunal prey invertebrates. Epiphytes provide a large portion of the primary production in seagrass habitats and are a trophic resource for seagrass invertebrates (Bologna and Heck 1999). The positive influence of epiphyte biomass on epifaunal prey invertebrate abundance we found in our study contradicts some findings in eelgrass beds (eg. Knight et al. 2015), but not in other seagrass species (eg. *Thalassia testudinum* beds in Florida (Hall and Bell 1988, Bologna and Heck 1999), and *Posidonia oceanica* in the Mediterranean

(Castejón-Silvo et al. 2012)). Inconsistent results could be due to the fact that eelgrass invertebrates exhibit top-down control on epiphytes (Whalen et al. 2013, Michel et al. 2015), meaning invertebrate abundance may not always follow a simple linear relationship with epiphyte biomass. The influence of year on prey invertebrate abundance in the negative binomial model also indicated interannual variability, which was also expected given eelgrass epifaunal invertebrates display yearly and seasonal fluctuations (Heck and Orth 1980).

Because invertebrates display species-specific responses to seagrass structure (Schneider and Mann 1991), we hypothesized that prey invertebrate communities in eelgrass habitat would have increased eelgrass-associated species compared to sediment associated species, with increasing eelgrass shoot density. Shoot density was the strongest predictor of prey invertebrate communities in the CAP, with communities in high eelgrass shoot density samples clustering away from those in low and medium eelgrass shoot density samples. This clustering, however, only suggests that prey invertebrate communities in high eelgrass shoot density quadrats were more speciose than in low and medium eelgrass shoot quadrats. Because greater than 80% of the variation in epifaunal prey invertebrate communities remained unexplained by the input variables in our study, the results from the CAP model do not support our hypothesis that eelgrass shoot density strongly influences epifaunal prey invertebrate community structure. This may suggest that eelgrass prey communities are largely stable across eelgrass density. Some studies, however, have found compositional changes in eelgrass invertebrate communities in response to other eelgrass metrics (eg.

Sutherland et al. (2013) found a positive association of caprellids to leaf area index, Sirota and Hovel (2006) found increasing crustacean biomass with eelgrass surface area, and Moore and Hovel (2010) found higher abundances of gammarids and caprellids with proximity to eelgrass patch edge (Moore and Hovel 2010). This suggests that eelgrass shoot density, epiphyte biomass, and year are not the only important variables influencing invertebrate communities. As previously discussed, a cascade of factors can influence faunal communities in eelgrass beds, including epiphyte species, eelgrass patch size and location, fish communities, and freshwater input, as well as species-specific responses to eelgrass density. Along with differing sampling methods, these variables could account for any differences in results among studies, suggesting that comparisons between studies require more standardized, comprehensive research methods.

Though eelgrass beds exhibit spatial heterogeneity, making comparisons across eelgrass ecosystems difficult (Bostrom et al. 2006), a vast body of literature supports the general patterns observed in this study. Our results suggest that decreasing eelgrass shoot density has a negative effect on epifaunal prey invertebrate abundance. Efforts should be made to maintain healthy beds of eelgrass in order to promote near-shore habitat productivity. This is especially so given suggestions that invertebrate densities are significantly decreased below a certain threshold of eelgrass density (Reed and Hovel 2006). If eelgrass beds are damaged, however, these reduced eelgrass areas still have ecological importance. While abundances of invertebrates will be depleted, the community composition of epifaunal invertebrate communities may be resilient to

eelgrass loss (barring possible differences in communities at a lower taxonomic resolution than assessed in this study). This is important to consider when assessing the value of eelgrass habitat. Eelgrass beds interspersed along the coastal Salish Sea for example, are characterized as patchy and sparse (Wright 2014). The habitat value of these beds, however, may not be lower than continuous or dense beds, given that the presence of eelgrass, either sparse or dense, hosts an invertebrate community that is potentially unique from other near-shore habitats. Even sparse beds will be important for marine fish species that potentially target eelgrass-associated invertebrates. Furthermore, eelgrass transplants that only have mild shoot success can still restore habitat function to an eelgrass-depopulated area.

#### Prey availability – Infaunal eelgrass invertebrates

Though we found a variety of potential salmon prey in infaunal eelgrass samples in the Comox Estuary, including harpacticoid copepods, tanaids, polychaete worms, and gammarid amphipods, our hypothesis that abundance of infauna in the Comox Estuary would increase with increasing eelgrass shoot density was not supported by the data. Both total infaunal invertebrate abundance and infaunal community composition were not affected by eelgrass shoot density, sediment core depth, or the percent of fine sediment <125 $\mu$ m. As most small invertebrates concentrate in the upper 5cm of sediment (Hines and Comtois 1985), a strong effect of sediment depth was not expected. However, other studies focusing on eelgrass sediment invertebrates have found increased infaunal abundances with increasing eelgrass shoot density (Bostrom and Bonsdorff 1997), and differing infaunal communities associated with changes in

above ground eelgrass biomass (Herkül and Kotta 2009). The lack of a clear relationship in our study could be related to our relatively low taxonomic resolution, or the low total abundance of eelgrass invertebrates in intertidal sediments in the Comox Estuary (an average of 1110 invertebrates/m<sup>2</sup> compared to over 10,000/m<sup>2</sup> in Bostrom and Bonsdorff (1997)). Despite relatively low abundances of invertebrates, sediments contained many invertebrates consumed by juvenile salmon, indicating that sediments may still be an important source of invertebrates for juvenile salmon in the Comox Estuary.

#### Prey availability - Zooplankton tows

Our hypothesis that eelgrass associated prey extended into the water column was not supported by the data. While many species appeared to be more abundant in tows over eelgrass within the estuary, compositional data were variable. All three tow types overlapped in the zooplankton CAP, indicating that the presence of eelgrass did not have a strong influence on invertebrates in the water column. Eelgrass associated invertebrates such as gammarid amphipods, harpacticoid copepods, and polychaete worms were not abundant in the zooplankton. Based on their absence in zooplankton tows during the day in 2015, and in invertebrate sweeps during the day and evening in 2016, eelgrass invertebrates in the Comox Estuary likely do not drift from eelgrass shoots into the water column, at least during the times sampled. This absence was unexpected, given the highly mobile nature of epifauna such as harpacticoid copepods (Mascart et al. 2015), isopods, and amphipods (Arponen and Bostrom 2011). Instead of migrating through the water column, epifauna in the Comox Estuary may migrate

closer to the sediment bottom, explaining why they were not collected in zooplankton tows or sweeps (Mascart et al. 2015). The absence of eelgrass invertebrates in the water column indicates that the presence of eelgrass does not influence zooplankton availability directly above eelgrass habitat. Therefore, any eelgrass invertebrates consumed by juvenile salmon are likely a result of fish feeding directly in eelgrass habitats or close to the sediment, rather than grazing on invertebrates in the water column as we suspected from our anecdotal observations of Chinook and Chum salmon.

#### Juvenile salmon diet – Stomach contents

Both species of juvenile salmon had a variety of prey items in their diets. Prey accumulation curves indicated that juvenile Chum salmon diets were adequately sampled, but not juvenile Chinook salmon diets (Cook and Bundy 2010). Terrestrial insects were present in both salmon species' diets, with dipterans occurring in high frequencies in juvenile Chum salmon diets and ants present in many juvenile Chinook salmon diets. Insects are a relatively high-energy prey item (Duffy et al. 2010), and are established resources for juvenile Chum salmon (Feller and Kaczynski 1975, Romanuk and Levings 2005, Sturdevant et al. 2012), and juvenile Chinook salmon (Brodeur 1989, Duffy et al. 2010, Hertz et al. 2015). In addition to insects, juvenile Chum salmon diets included a wide variety of prey, but were dominated by harpacticoid copepods and small polychaete worms by prey counts, weights, and frequency of occurrence. Benthic invertebrates such as these made up over 70% of juvenile Chum salmon diet by count. The harpacticoid-dominated, largely benthic diet found here is

consistent with diets of juvenile Chum salmon of similar sizes that were collected between April and May (mean fork length 42mm) in the Nanaimo Estuary (Healey 1979) and within Puget Sound (Feller and Kaczynski 1975 (fork length 30-59mm), Simenstad and Cordell 2000 (fork length 40-50mm)). Benthic productivity may therefore have important implications for the growth of juvenile Chum salmon.

Juvenile Chinook salmon diet was more planktonic than juvenile Chum salmon diet, with planktonic species, mainly decapod shrimp and crab megalopae, making up 30% of juvenile Chinook salmon diets by count. Juvenile Chinook salmon diets were also closely associated with many planktonic species in the CAP, including crab megalopae, crustacean nauplii, barnacle cyprids, decapod shrimp, and hyperiid amphipods. Juvenile salmon length, relative weight, digestion score, and gut fullness did not seem to greatly explain the diet of either species. Like juvenile Chum salmon diets, juvenile Chinook salmon diets were also dominated by harpacticoid copepods by count, as seen also in the Nanaimo Estuary (Healey 1991). The majority of harpacticoid copepods in the epifaunal and infaunal eelgrass samples, however, were darker in colour than those found in juvenile salmon diets, potentially indicating species differences. Average length of harpacticoid copepods in juvenile Chum salmon diets was significantly larger than for harpacticoid copepods found in epifaunal and infaunal eelgrass samples or for cyclopoid copepods in zooplankton. This indicates that juvenile Chum salmon were likely targeting a larger average size of harpacticoid copepod than offered in any of the prey sources assessed.

Average size and colour differences in harpacticoid copepods consumed by juvenile Chum salmon compared to potential sources make it difficult to pinpoint exactly from which habitat these invertebrates originated. There were, however, some epifaunal eelgrass harpacticoids that looked visually similar to those found in juvenile salmon diets and our epifaunal eelgrass samples were the only samples in the Comox Estuary with an abundance of harpacticoid copepods. This indicates a high probability that juvenile salmon were selecting their harpacticoid copepods from eelgrass.

Additionally, eelgrass has been found to host unique harpacticoid copepod assemblages known to be important for juvenile Chum salmon (Sanborn et al. 1988).

#### Juvenile salmon diet – Stable isotope analysis

Stable isotope analysis strengthened our conclusion that juvenile Chum salmon in the Comox Estuary were reliant on eelgrass productivity for foraging. Comparing the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values of juvenile salmon to potential diet sources, we estimated that eelgrass invertebrates made up approximately 80% of the diets of juvenile Chum salmon.

Juvenile Chum salmon from our study were slightly more enriched in  $\delta^{13}\text{C}$  than those of similar sizes in Howe Sound ( $-15.15 \pm 1.82$  compared to  $-19.35 \pm 1.07$ , respectively) (Romanuk and Levings 2005). The  $\delta^{15}\text{N}$  values of juvenile Chum salmon from both areas, however, were similar ( $12.53 \pm 0.73$  compared to  $12.9 \pm 1.16$ ) (Romanuk and Levings 2005). The  $\delta^{13}\text{C}$  enrichment of juvenile Chum salmon in our study relative to those in Howe Sound may be explained by the presence of eelgrass in the Comox Estuary since enriched  $\delta^{13}\text{C}$  signatures from eelgrass ecosystems can transfer into meiofauna and higher order consumers (Ha et al. 2013). The absence/sparseness of

eelgrass in Howe Sound (Wright 2014) and the high shoot density of eelgrass in the Comox Estuary could explain higher  $\delta^{13}\text{C}$  enrichment in juvenile Chum salmon and its epibenthic prey in the Comox Estuary.

Overall, the  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  signatures of juvenile Chum salmon closely reflected those of eelgrass epifaunal prey invertebrates, suggesting eelgrass is an important foraging habitat for juvenile salmon. The  $\delta^{13}\text{C}$  isotope signatures from epifaunal prey invertebrates in our study, however, were only compared to zooplankton and terrestrial invertebrates, not to benthic invertebrates outside of eelgrass habitat. Without establishing that benthic invertebrates inside and outside of eelgrass habitat reflect different isotopic signatures, some uncertainty remains as to whether juvenile Chum salmon were feeding directly on eelgrass invertebrates or simply on all benthic invertebrates.

### Implications

Juvenile salmon use near shore habitat extensively during the early marine period (Duffy et al. 2005), which is a critical time for salmon growth, with larger, faster growing fish having increased probabilities of surviving to adulthood (Beamish et al. 2003, Duffy and Beauchamp 2011). While mechanisms that determine growth in estuarine environments are poorly understood, the capacity of these habitats to provide prey is likely an important component of salmon survival (Simenstad and Cordell 2000). In our study, juvenile Chum salmon and juvenile Chinook salmon diets were dominated by harpacticoid copepods, which were found in abundance in epifaunal and

infaunal eelgrass samples. Juvenile Chum salmon also displayed stable isotope signatures that imply the use of eelgrass habitat for foraging opportunities. The presence of eelgrass is therefore an important source of invertebrate productivity for juvenile salmon since it directly influences the availability of benthic prey. Eelgrass habitat also indirectly supports benthic productivity via the transfer of carbon through the benthic food web (Ha et al. 2013). While dense eelgrass beds support increased epifaunal prey invertebrate densities, our study found that sparse eelgrass plays host to the same invertebrate compositions as dense eelgrass. All eelgrass beds may therefore provide unique invertebrate communities that add foraging value to near shore areas. Conservation of eelgrass habitat, especially near salmon bearing streams, thus should be a management priority. The value of even a sparse amount of eelgrass in providing foraging opportunities should also not be overlooked.

## **SUMMARY**

We described infaunal and epifaunal prey invertebrate communities and abundance across a gradient of eelgrass shoot density in order to understand how eelgrass affects prey availability for juvenile salmon. However, eelgrass communities can be influenced by a multitude of factors that were not addressed in this study, including proximity to patch edge, water currents, salinity, and degree of wave and sun exposure. It is important to keep in mind that our simple model does not capture all of the variability in eelgrass prey invertebrate abundance, and much opportunity exists for further research relating these factors to the capacity of eelgrass to supply prey for juvenile salmon. Identifying species down to finer taxonomic resolutions may also

reveal patterns in infaunal and epifaunal prey invertebrates that we were not able to describe with the taxonomic groups used in this study. While we feel that our results adequately reflect juvenile salmon feeding habits in the Comox Estuary given that our sampling locations were the only places where juvenile salmon were caught in high abundances, we acknowledge that juvenile salmon diets can be highly variable, differing between estuaries and even locations within an estuary. While Chinook salmon diets were all caught from one location in the estuary, their consumed prey did not reach an asymptote, indicating that juvenile Chinook salmon diets may not have been adequately described and may need more evaluation before strong conclusions on dietary habits are drawn. Future studies should consider the variability of juvenile salmon diets and ensure multiple locations, tows, and individuals are sampled to capture all of this variability.

Overall, our study shows that eelgrass habitat and shoot density are important for juvenile salmon and that eelgrass conservation and protection should be prioritized. Eelgrass habitat directly and indirectly increases access to benthic invertebrates, providing forage communities composed mainly of harpacticoid copepods, gammarid amphipods, and polychaete worms. These benthic invertebrates are important sources of food for juvenile salmon, which we showed through both gut content and stable isotope analysis. In order to safeguard juvenile salmon access to productive foraging grounds, eelgrass habitat should therefore be protected. Diet composition of juvenile salmon in this study is consistent with studies from the Nanaimo Estuary and Puget Sound, both of which have eelgrass habitat throughout (Healey 1991, Mumford 2007).

Comparing diets of juvenile salmon and prey availability in estuaries with and without eelgrass may give further insight into the role of eelgrass habitat in providing important foraging opportunities. The need for such protection and further research is underscored by the fact that the growth and survival during the early marine period is a powerful determinant of overall return rates, and that foraging opportunities may affect this growth and survival (Duffy et al. 2000).

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## **Chapter 3: Management Relevance**

Our study showed that juvenile salmon residing in the Comox Estuary rely substantially on benthic invertebrates. These benthic invertebrates are positively influenced, both directly and indirectly, by the presence of eelgrass in the estuary. Higher prey availability promotes faster growth in the early marine period, which is a critical time for growth in salmon life history. The capacity of eelgrass to increase foraging opportunities for juvenile salmon therefore indicates that eelgrass habitat is an important feature in the near-shore environment. The following chapter will provide a brief overview of the status of salmon and eelgrass populations and management in the Salish Sea, and will discuss the importance of managing eelgrass habitat in the context of juvenile salmon survival. This chapter will also suggest future research opportunities.

### **SALMON POPULATIONS IN THE SALISH SEA**

Management of natural resources is often hindered by uncertainty. This uncertainty can originate from a variety of sources, including the difficulties of managing multiple stakeholders and their respective, often conflicting, interests, and uncertainty in predicting responses of resources that are part of ever-changing, complex ecological systems (Ascough 2008). Pacific salmon management provides a prime case study of the uncertainties that can befall management. Multiple stakeholders have a vested interest in Pacific salmon management, including aboriginal peoples, environmental

groups, and the variety of individuals involved in the fisheries industry (Hamouda et al. 2005). Salmon are also important to the public (Gende et al. 2002), meaning decisions pertaining to salmon management are more difficult to make, given they may be faced with increased public scrutiny. Additionally, salmon are intrinsically linked to both marine and terrestrial systems (Gende et al. 2002). Given that decisions involving watersheds or forestry, for example, can have implications for salmon health, proper salmon management requires an integrative approach (Gende et al. 2002). On top of the necessity for communication between governmental departments, Pacific salmon also cross international boundaries in both the marine and freshwater phase, and management is therefore complicated by the need for cooperation between the United States and Canada (Yanagida 1987). All of these management challenges are compounded by the fact that salmon have complex life histories (Healey 1991), the understanding of which limits the ability of managers to predict salmon returns (Beamish et al. 2003).

Challenges surrounding salmon management are addressed in Canada through two main bodies of work, the Policy of the Conservation of Wild Pacific Salmon (DFO 2005), and the Pacific Salmon Treaty (Yanagida 1987). The overarching goal of the Policy of the Conservation of Wild Pacific Salmon is to restore and maintain healthy populations of wild Pacific salmon (DFO 2005). The policy acknowledges the variety of stakeholders concerned with the management of Pacific salmon, and commits to designing open management plans that take the interests of all parties in to consideration (DFO 2005). Accounting for the ecosystem-wide effect that Pacific

salmon can have, the Policy of the Conservation of Wild Pacific Salmon also outlines an integrated management approach that involves multiple levels of government and an assessment of the linkages between salmon and the watersheds from which they originate (DFO 2005). Issues arising from the transboundary nature of Pacific salmon are addressed in the Pacific Salmon Treaty (Yanagida 1987). The agreement negotiates harvest sharing between the two countries, and also outlines the roles that Canada and the United States play in monitoring and sustaining Pacific salmon populations originating within their respective boundaries (Yanagida 1987). While multiple efforts are in place to address the challenges of managing Pacific salmon populations, the uncertainty around important stages of their life history remains an unaddressed challenge poses management limitations, particularly in the Salish Sea (Beamish et al. 2003).

Many wild populations of Pacific salmon in the Salish Sea are severely depleted (Beamish et al. 2003). The Puntledge River Chinook salmon for example, which were once one of the most commercially important stocks of Chinook salmon in British Columbia, have declined to less than 50% of their historical abundance (Trites et al. 1996). Efforts to rebuild these populations to previous levels via the release of over two million Chinook salmon annually over the last ten years have largely failed (Puntledge Hatchery- unpublished data). Other supplementary efforts in the Salish Sea have also largely failed to rebuild or maintain Chinook populations. Harvest rates in the Strait of Georgia, for example, declined drastically from over 750,000 individuals annually in the 1970s, to an average of 168,000 from 1987-1991 (Beamish et al. 1995).

The story is much the same for Coho salmon, with low returns of both species persisting across the Strait of Georgia (Beamish et al. 2003). While Chinook salmon and Coho salmon are focal species for conservation due to their drastically declined abundances (Riddell 2015), the health of other species of Pacific salmon in the Salish Sea also warrants attention. Two ecologically significant units of Chinook salmon and Coho salmon, two ecologically significant units of Chum salmon, and three of Sockeye salmon are listed as a species of concern in the Salish Sea (Gaydos and Brown 2011). As low returns of Pacific salmon in the Salish Sea are largely attributed to the early marine period, protecting habitat that is important to the early marine period is paramount (Beamish et al. 2003). The fact that little information about early marine habitat is available underscores the need for management practices to be sensitive to unknowns, and take preemptive action in habitat conservation. This is furthered by the fact that large scale changes are predicted in the Salish Sea ecosystem in the near future, including loss of estuarine habitat due to sea level rise, increased development and pollution, and shifts in ocean regimes (Johannessen and Macdonald 2009).

#### **EELGRASS HABITAT IN THE SALISH SEA**

Loss of eelgrass habitat is a global concern (Orth et al. 2006), with total eelgrass cover shrinking by 1.4% per year (Short et al. 2010). While many habitat types are vulnerable to human impacts, loss of eelgrass habitat is especially noteworthy due to its capacity as an ecosystem engineer (Bos et al. 2007), and the disproportionate effect it has on the marine food webs (Plummer et al. 2013). Eelgrass is also an important cultural and economic species for many indigenous peoples across the North West

coast of British Columbia (Cullis-Suzuki et al. 2015); while the traditional practice of collecting eelgrass in the early spring for its sweet, protein and nutrient rich rhizomes is not widely observed today (Cullis-Suzuki et al. 2015), eelgrass is still utilized in the harvest of herring spawn (Thornton et al. 2010). Eelgrass faces numerous simultaneous threats, including direct habitat development, reductions in water clarity, nutrient loading, and wasting disease (Short et al. 2010). Sea level rise due to climate change is also expected to decrease eelgrass habitat range (Plummer et al. 2012). Acknowledging eelgrass as an important ecological and cultural resource, a number of non-profit groups spearhead the monitoring and conservation of eelgrass across the coast, including the Community Mapping Network, the Seagrass Conservation Working Group, and the SeaChange Conservation Society (Boyer et al. 2009). These stewardship groups work closely with local conservation societies and First Nations to identify areas where monitoring, mapping, and restoration of eelgrass habitat is needed (Boyer et al. 2009). Via this network, a large sum of knowledge of the extent of eelgrass in the Salish Sea has been amassed, providing baseline information that serves as the first step towards proper management of eelgrass habitat (Wright et al., 2014). The Department of Fisheries and Oceans also recognizes the importance of eelgrass, acknowledging that it meets the requirements for an ecologically significant species (DFO 2009). Eelgrass in Canada is considered fish habitat, and is therefore protected by the Fisheries Act under the policy of no net loss of fish habitat (Fisheries Act 1985). Activities that result in significant damage to eelgrass beds, therefore often require compensatory action (DFO 2002). Eelgrass plantations, however, have had variable success rates and are not monitored for sufficient amounts of time to ensure stability of

transplanted sites (DFO 2002). The fact that eelgrass habitat is continually deteriorating at a rapid rate, especially in North America (Short et al. 2010) lends to the argument that the habitat should be assessed for its value towards species that inhabit it, especially those as ecologically, culturally, and economically important as Pacific salmon.

## **MANAGEMENT IMPLICATIONS AND FUTURE RESEARCH**

While many expected changes to the Salish Sea are difficult to control, managers have the ability to influence the degree of habitat destruction through management of coastal development and other human activities (Johannessen and Macdonald 2009). Habitat conservation is identified as a crucial step in rehabilitating wild Pacific salmon populations in British Columbia (DFO 2005). Results from this study suggest that such habitat conservation should take the importance of eelgrass into account. Eelgrass shoot density influences the productivity of ecosystems by increasing habitat for eelgrass-associated invertebrates. Isotope analysis of juvenile Chum salmon, and stomach content analysis of both Chum salmon and Chinook salmon juveniles indicated a high contribution of benthic invertebrates in the diets of both species. Though juvenile salmon may not have been feeding on the most common benthic invertebrates in intertidal eelgrass habitat, the high abundance of harpacticoid copepods in eelgrass epifaunal eelgrass samples compared to sediments and zooplankton, as well as the overwhelming presence of harpacticoid copepods in juvenile salmon diets, indicates that eelgrass is an important habitat feature that provides foraging opportunities for juvenile salmon in the early marine period. As prey

communities of eelgrass do not appear to change drastically across a gradient of intertidal eelgrass shoot density, even a minimal presence of eelgrass in an estuary may have a positive influence on juvenile Pacific salmon that feed on eelgrass invertebrate communities. While both Pacific salmon and eelgrass habitat are important separately, the association between them can motivate conservation efforts that benefit both eelgrass habitat and Pacific salmon.

Given the importance of eelgrass habitat towards prey availability of juvenile salmon, salmon management regimes should embrace eelgrass habitat as an important component of the ecosystem continuum through which salmon migrate, and should consider eelgrass conservation as an avenue to maintain near-shore habitat health for juvenile salmon. Eelgrass conservation, on the other hand, should consider juvenile salmon as one of the critical species benefitting from the conservation of eelgrass habitat, and should involve stakeholders associated with juvenile salmon in eelgrass conservation. An important first step in this endeavor is to continue mapping and monitoring eelgrass habitat. Knowledge of eelgrass range and density across the coast will help determine the access juvenile salmon have to eelgrass habitats. These assessments can also help to provide a baseline of eelgrass habitat, allowing managers to track responses of eelgrass habitat to anthropogenic pressures.

Juvenile salmon management, and eelgrass habitat conservation could then work simultaneously on projects in the near shore environment that would benefit both juvenile salmon and eelgrass habitat. For example, log boom storage in estuaries can

shade out eelgrass habitat, and also provide refuge for harbor seals, a major predator of out-migrating juvenile salmon (Yurk and Trites 2000). Removal of the log booms, and remediation of underlying habitat may be an avenue to decrease salmon predation, increase eelgrass coverage, and boost the productivity of the near-shore environment that juvenile salmon rely on. Restoration of marsh habitat may also provide increased refuge and terrestrial food sources for juvenile salmon, while simultaneously decreasing sediment runoff and potentially offering protection for eelgrass habitat by trapping sediments.

In order to better understand the role of eelgrass towards survival of juvenile salmon, the abundance of eelgrass habitat in near-shore areas, and the proximity of eelgrass to salmon bearing streams and locations where juvenile salmon congregate, could then be related to growth and survival of juvenile salmon during the early marine stage. While many studies have compared invertebrate productivity in eelgrass beds to that in unvegetated sediments, future studies should also compare the relative productivity of estuaries with differing densities of eelgrass, and relate the survival of salmon in those estuaries to eelgrass health. Such research would allow for increased understanding of how the presence of eelgrass influences estuarine productivity as a whole, which can determine how long juvenile salmon reside in estuaries (Healey 1991). Similarly, diet comparisons of juvenile salmon residing in eelgrass populated, and eelgrass unpopulated estuaries can further our understanding of how these habitats influence juvenile salmon foraging success.

While some successful eelgrass restoration projects result in expanding net coverage of eelgrass (DFO 2002), special consideration should be given to construction around eelgrass beds close to salmon bearing streams. Based on the premise that eelgrass habitat is important for juvenile salmon foraging, compensating for the destruction of eelgrass beds utilized by juvenile salmon by artificially creating beds in alternate locations will not offset the negative impacts of near-shore development for juvenile salmon. Finally, it is important to note that while eelgrass prey invertebrates increased with increasing eelgrass shoot density, the prey communities at the taxonomic resolution assessed in this study were not largely influenced by eelgrass shoot density. While loss of low eelgrass shoot density habitat may not have the same effect on near-shore productivity as the loss of high shoot density eelgrass habitat, loss of any eelgrass habitat should not be taken lightly, as even sparse eelgrass supports a high abundance of invertebrates and potentially unique invertebrate communities.

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## Appendix

### SUPPLEMENTARY TABLES

Table S1. Average and standard deviation (sd) of eelgrass invertebrate counts in low, medium, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015-2016. Averages are rounded to the nearest whole number.

Taxon	Low (0-49 shoots/m <sup>2</sup> )		Medium (50-99 shoots/m <sup>2</sup> )		High (100+ shoots/m <sup>2</sup> )	
	2016	2016	2015	2016	2015	2016
	Average(sd)	Average(sd)	Average(sd)	Average(sd)	Average(sd)	Average(sd)
<b>Annelida</b>						
Small Polychaete worms (<5cm)	1.1(2.2)	0.5(0.5)	2.9(4.1)	1.3(2.4)	5.9(8.8)	2.5(5.2)
<b>Gastropoda</b>						
Snails*	0.6(0.9)	1.0(1.1)	3.0(4.3)	3.0(4.0)	4.4(4.5)	1.3(2.3)
<b>Crustacea</b>						
Harpacticoid copepods	182.9(287.8)	144.4(90.8)	297.1(306.5)	472.2(577.4)	388.2(558.4)	1178.5(1196.2)
Limpets*	4.8(11.9)	0(0)	12.5(13.4)	0.1(0.3)	22.3(25.6)	0.2(0.4)
Barnacles*	0.1(0.4)	35.3(51.4)	6.5(18.4)	149.8(124.0)	4.2(6.6)	482.5(425.0)
Crabs*	0.1(0.3)	0(0)	0.1(0.3)	0(0)	0(0)	0(0)
Mussels*	0.3(0.7)	0(0)	5.1(14.0)	0.2(0.4)	5.1(7.8)	1.5(2.3)
Mites	5.5(13.0)	0.1(0.3)	5.1(8.5)	0.1(0.3)	16.4(33.9)	2.6(3.8)
Barnacle cyprids*	0(0)	0.7(0.9)	0(0)	0.3(0.7)	0(0)	2.8(6.4)
Shrimp zoea*	0.1(0.3)	0(0)	0(0)	0(0)	0(0)	0(0)
Decapod shrimp*	0(0)	0(0)	0(0)	0(0)	.21(0.9)	0(0)
Ostracods*	0(0)	0(0)	0(0)	0(0)	1.7(4.7)	0(0)
Crab zoea*	0(0)	0(0)	0(0)	0(0)	0(0)	0.5(2.)
Crab megalons*	0(0)	0(0)	0(0)	0(0)	0.3(0.9)	0(0)
Euphasiid zoea*	0(0)	0(0)	0.2(0.7)	0(0)	0(0)	0(0)
Cladocerans*	0(0)	0.5(1.4)	0(0)	0(0)	0(0)	0(0)
<b>Amphipoda</b>						
<b>Malacostraca</b>						
Corophid amphipods	2.0(3.0)	0.1(0.3)	9.1(16.0)	1.0(1.9)	12.3(12.9)	6.6(4.1)
Tanaids	0(0)	0(0)	0(0)	0.1(0.3)	0(0)	0.5(1.8)
Small isopod (<1cm)	0(0)	0(0)	0(0)	0.1(0.3)	0.1(0.3)	0.5(2.1)
Large isopod (>1cm)	0.2(0.4)	0(0)	0.7(1.4)	0.2(0.4)	1.5(1.5)	0.9(2.0)
Cumaceans	0.1(0.3)	0.2(0.4)	1.1(2.8)	1.3(3.8)	2.3(2.8)	0.9(2.1)
<b>Senticaudata</b>						
Gammarid amphipods	7.0(9.4)	1.6(3.0)	33.9(43.0)	4.7(6.0)	69.9(88.4)	7.4(11.62)
Other senticaud amphipods	0.1(0.5)	0(0)	1.0(2.8)	0.5(1.0)	1.4(3.0)	0.7(1.5)
Other amphipods	0(0)	0(0)	0(0)	0(0)	0.4(1.8)	0.1(0.3)
<b>Insecta</b>						
Dipterans*	0(0)	0.1(0.3)	0(0)	0(0)	0(0)	0(0)

\*Data removed for analysis of epifaunal prey invertebrates in eelgrass

Table S2. Summary of negative binomial model of prey invertebrate abundance, with apparent outlier removed, in eelgrass quadrats from the Comox Estuary, British Columbia, May 2015-2016.

	Estimate	SE	Z	P
Intercept	4.854136	0.227944	21.295	<0.0001
Year*	0.653732	0.267468	2.444	0.0145
Shoots collected*	0.120007	0.025131	4.775	<0.0001
Epiphyte biomass (mg/shoot)*	0.006371	0.001738	3.666	0.0002

Null deviance: 116.794 on 73df, residual deviance: 89.655 on 70df, 2 x log-likelihood: -1017.245

\*Data are centered

Table S3. Results of model selection of eelgrass variables to predict epifaunal invertebrate abundance per 0.0625m<sup>2</sup> quadrat using a negative binomial model. All variables have been standardized to unit variance with a mean of 0.

Coefficients						df	LogLikelihood	AIC	Delta AIC
(Intercept)	Year	Epiphyte Biomass	Shoot density/0.0625m <sup>2</sup>	Eelgrass Surface Area (cm <sup>3</sup> )	Shoot density/0.125m <sup>2</sup>				
4.770	0.779	0.007	0.199	0.000	NA	6	-517.997	1047.994	0.000
4.786	0.703	0.006	0.136	NA	NA	5	-519.102	1048.205	0.210
4.849	0.753	0.007	0.173	NA	-0.013	6	-518.712	1049.425	1.430
4.810	0.808	0.007	0.220	0.000	-0.008	7	-517.824	1049.648	1.654
5.179	0.594	0.005	NA	NA	0.026	5	-524.976	1059.953	11.959
5.290	0.714	0.005	NA	0.000	NA	5	-524.995	1059.990	11.996
5.099	0.627	0.005	NA	0.000	0.016	6	-524.178	1060.356	12.362
5.925	0.890	0.004	NA	NA	NA	4	-529.041	1066.082	18.087

Table S4. Average and standard deviation (sd) of shoots/m<sup>2</sup> and average, standard deviation, and frequency of occurrence (FO) of infaunal invertebrate counts in 10cm deep, 8cm diameter sediment cores from quadrats in low (n=11), medium (n=5), and high (n=13) eelgrass shoot density categories in the Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.

Taxon/Measure	Low (0-49 shoots/m <sup>2</sup> )		Medium (50-99 shoots/m <sup>2</sup> )		High (100+ shoots/m <sup>2</sup> )	
	Average count(sd)	FO (sd)	Average count(sd)	FO (sd)	Average count(sd)	FO (sd)
Average shoots/m <sup>2</sup>	32(16)		86(14.3)		233(99.5)	
Annelida						
Small polychaete worms (<5cm)	17(12.8)	81.8(0.4)	27(29.4)	100.0(0)	30(54.9)	83.3(0.4)
Other	2(2.8)	45.4(0.5)	4(3.2)	83.3(0.4)	2(2.3)	50.0(0.5)
Crustacea						
Harpacticoid copepods	42(96.4)	63.6(0.5)	7(4.6)	100.0(0)	29(54.4)	66.7(0.5)
Barnacle cyprids*	0.2(0.5)	8.3(0.5)	0(0)	0(0)	0(0)	0(0)
Crab megalopae*	0.2(0.5)	16.7(0.5)	0(0)	0(0)	0.1(0.5)	8.3(0.5)
Hermit crabs*	0(0)	0(0)	0(0)	0(0)	0.1(0.5)	8.3(0.5)
Amphipoda						
Malacostraca						
Corophid amphipods	3(3.4)	63.6(0.5)	6(6.6)	66.7(0.5)	1(1.8)	41.6(0.5)
Tanaids	9(19.9)	36.3(0.5)	3(5.8)	16.6(0.4)	2(7.2)	8.3(0.3)
Cumaceans	4(3.7)	63.6(0.5)	2(2.4)	83.3(0.4)	8(10.2)	50.0(0.5)
Senticaudata						
Gammarid amphipods	3(5.1)	45.4(0.5)	5(9.2)	66.7(0.5)	5(7)	50.0(0.5)

\*Data removed for analysis of infaunal prey invertebrates in eelgrass

Table S5. Pearson correlations between  $\delta^{13}\text{C}$  signatures, fork length, weight, and C:N ratios of juvenile Chum salmon caught in the Comox Estuary, British Columbia, May 2015.

	C:N	Fork Length	Weight
$\delta^{13}\text{C}$ Chum salmon	-0.11	0.09	0.11
Weight	-0.85	-	-

Table S6. Mean, standard deviation, and Bayesian credible intervals for estimated diet proportions of food sources to the diet of juvenile Chum salmon in Comox Estuary using uninformative and informative priors.

Source	Mean (%)(sd)		Bayesian credible interval (2.5-97.5)	
	Uninformative	Informative	Uninformative	Informative
Eelgrass invertebrates	79.8(10.2)	82.5(8.3)	56.4-95.2	6.2-94.5
Insects	4.2(3.4)	8.9(4.2)	0.1-12.6	2.0-18.0
Zooplankton	16.9(11.0)	8.6(9.0)	0.7-41.3	0.0-32.5

## SUPPLEMENTARY FIGURES

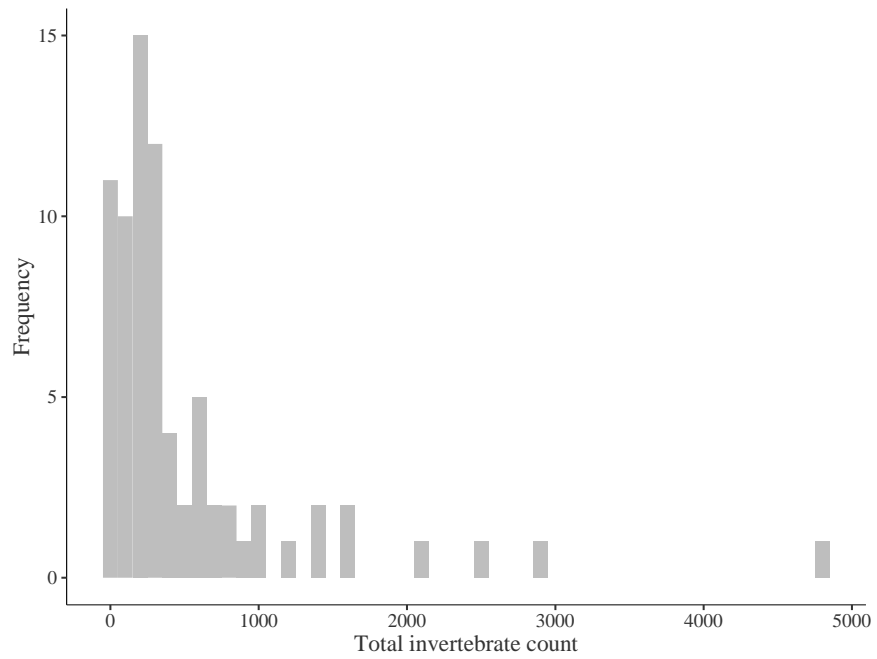


Figure S1. Histogram of total epifaunal prey invertebrate abundance in 0.0625m<sup>2</sup> eelgrass quadrats in Comox Estuary, BC, May 2015-2016.

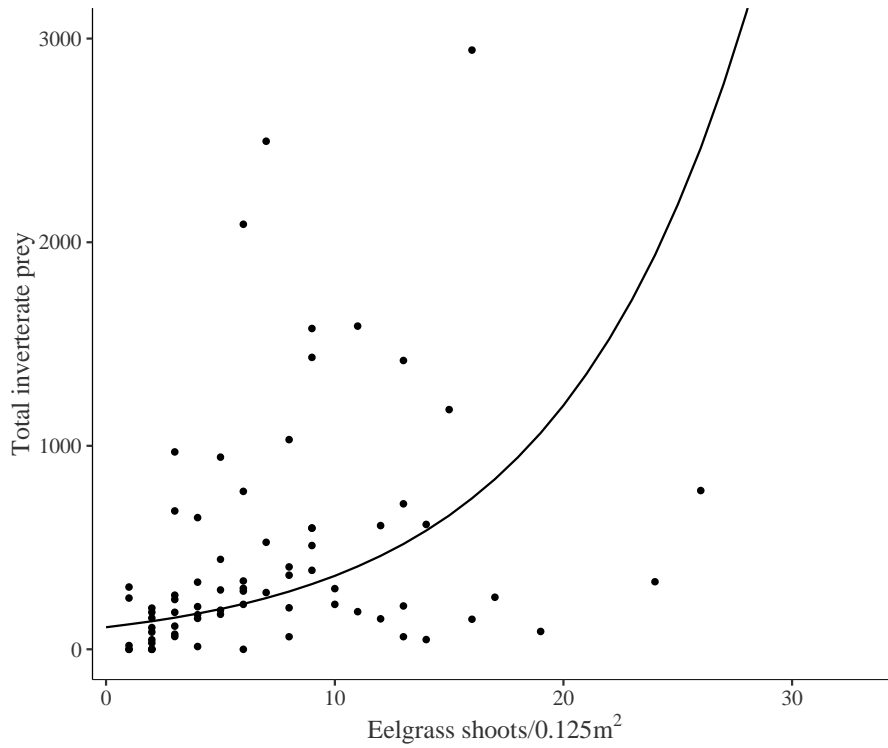


Figure S2. Predicted values of negative binomial model of epifaunal prey invertebrate abundance as a function of shoot density in 0.0625m<sup>2</sup> eelgrass quadrats collected in May 2015 and 2016 in the Comox Estuary, British Columbia. Year is held at its centered value, and epiphyte biomass at its mean, and a potential outlier was removed.

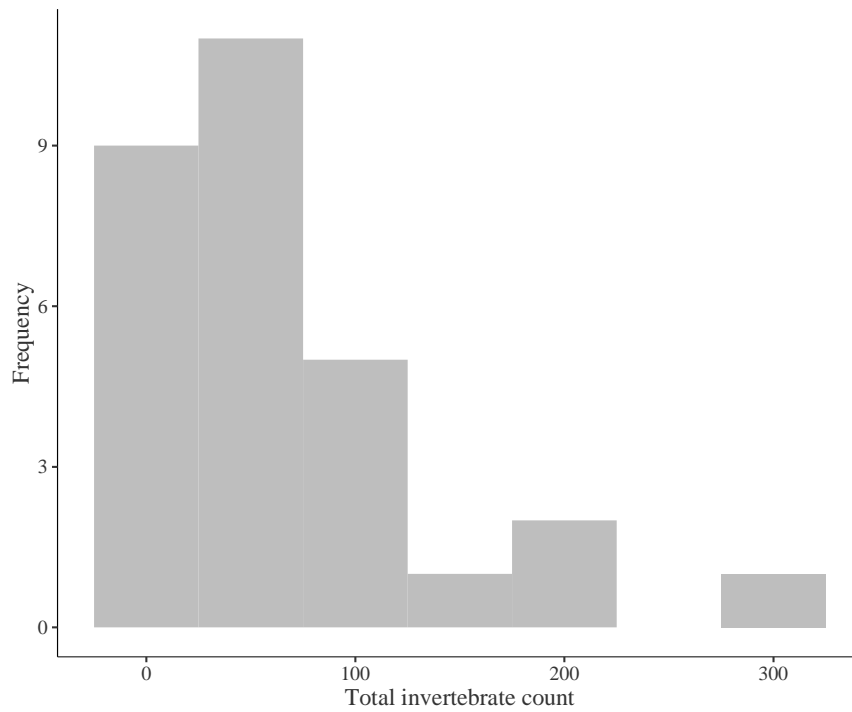


Figure S3. Histogram of total infaunal prey invertebrate abundance in sediment cores from low, medium, and high-density eelgrass quadrats in Comox Estuary, British Columbia, May 2015-2016.

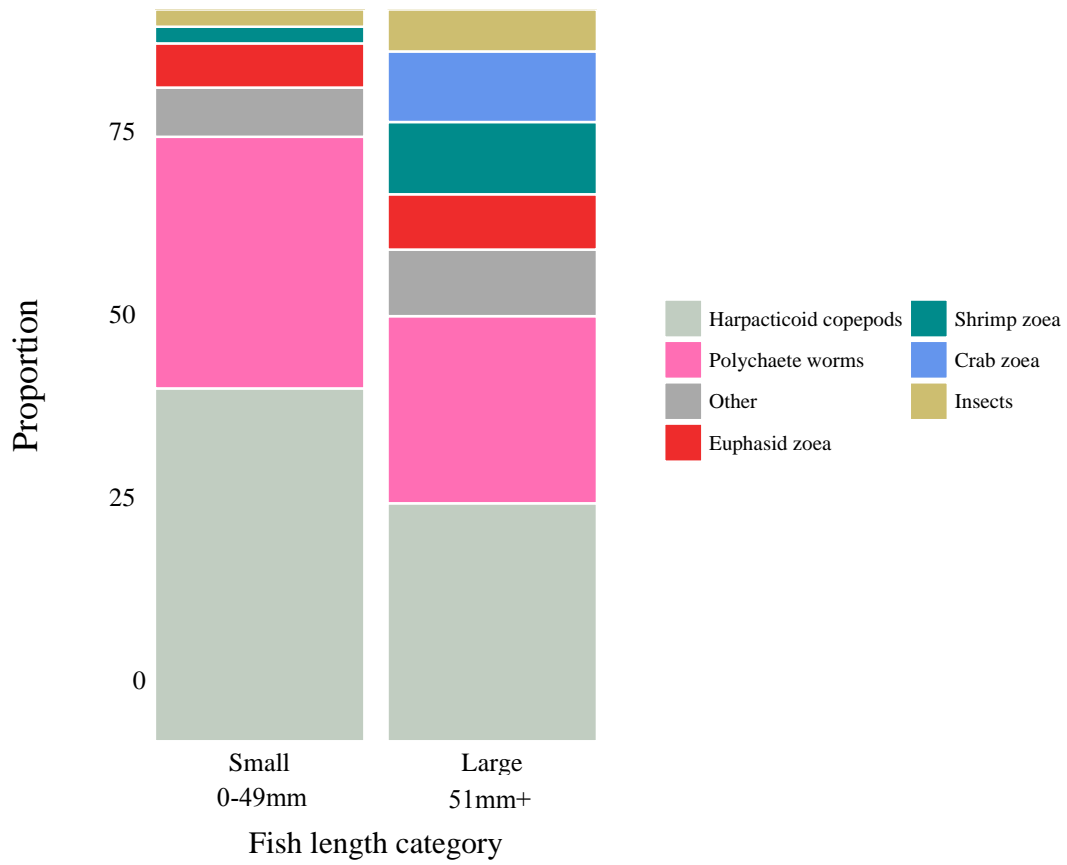


Figure S4. Average proportion of invertebrate weight in diets of small and large juvenile Chum salmon caught in the Comox Estuary, British Columbia, 2015.