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Eelgrass as Valuable Nearshore Foraging Habitat for Juvenile Pacific Salmon in the Early Marine Period

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Abstract

The early marine period is a critical life history stage for growth and survival of anadromous juvenile Pacific salmon. The integrity of nearshore ecosystems where juvenile salmon reside and the capacity of these habitats to provide prey can thus influence overall salmon returns. Eelgrass *Zostera marina* beds in particular are considered critical nearshore habitat. By examining how juvenile salmon use this habitat during their early marine life, we gain insight into the trophic dynamics in these ecosystems and help gauge the effects of accelerating eelgrass loss and nearshore habitat development on juvenile salmon. This project investigated the role of eelgrass as juvenile salmon foraging habitat. We compared juvenile Chum Salmon *Oncorhynchus keta* and juvenile Chinook Salmon *O. tshawytscha* diets to prey availability in zooplankton tows and in epifaunal eelgrass samples across a gradient of eelgrass density in the Comox Estuary, British Columbia. Harpacticoid copepods dominated the diets of both juvenile Chum Salmon and Chinook Salmon and were found in abundance in eelgrass blades. We complemented diet analysis with carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotope analysis to examine the relative contribution of zooplankton, eelgrass epifaunal invertebrates, and terrestrial invertebrates to the diet of juvenile Chum Salmon. Juvenile Chum Salmon isotope ratios closely reflected those of eelgrass invertebrates, suggesting that eelgrass invertebrates made up approximately 80% of the diets of juvenile Chum Salmon. Our results highlight the value of eelgrass in providing nearshore foraging opportunities for juvenile salmon and suggest that eelgrass habitat protection and restoration may provide critical support for growth, thereby easing the transition of juvenile salmon from freshwater to the marine environment.

Rapid growth of Pacific salmon *Oncorhynchus* spp. during their early marine phase correlates with survival to adulthood (Beamish and Mahnken 2001). Larger fish have more energy reserves to survive subsequent periods of low food availability (Beamish et al. 2004), and rapid growth reduces vulnerability to predation during early marine residence (Duffy and Beauchamp 2011). The capacity of nearshore ecosystems to provide prey and promote juvenile salmon growth can thus be critical to overall return

rates (Beamish et al. 2004). We have little understanding, however, of what habitat features support prey provision (Beamish et al. 2003). Estuaries are productive nearshore habitats potentially critical to juvenile salmon (Levings 2016); for example, Chum Salmon *O. keta* and Chinook Salmon *O. tshawytscha* fry introduced into less-modified estuaries display higher survival rates than smolts introduced into degraded estuaries (Magnusson and Hilborn 2003). Salmon display high growth rates in estuaries,

Subject editor: Kenneth Rose, University of Maryland Center for Environmental Science, Cambridge

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Received July 20, 2017; accepted December 11, 2017

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which requires an abundance of food (Neilson et al. 1985; Healey 1991). Seagrass beds supply valuable estuarine nursery habitat and food provision for many fisheries species (McDevitt-Irwin et al. 2016). Eelgrass *Zostera marina* is the primary native seagrass species in estuaries across North America. In the face of large-scale eelgrass loss in many Pacific estuaries (e.g., Tallis et al. 2009; Thom et al. 2012) and drastically diminished returns of Pacific salmon across the West Coast of North America (Noakes et al. 2000), it is critical to understand how eelgrass habitat affects juvenile salmon prey provision.

This study explored the relationship between eelgrass and nearshore foraging opportunities for juvenile Pacific salmon. The first objective was to understand how eelgrass shoot density influenced juvenile salmon prey, in terms of both prey abundance and community structure. We sampled eelgrass blades and sediments across a gradient of eelgrass density, hypothesizing that the structure of eelgrass would provide habitat for a variety of juvenile salmon prey. We also hypothesized that niche space would increase with eelgrass density, leading to increased invertebrate abundance, and that species assemblages in sparse eelgrass would have higher abundance of sediment-associated species such as tanaids (Holdich and Jones 1983), while assemblages in dense eelgrass would have higher compositions of eelgrass-associated species such as isopods (Boström and Mattila 1999). We also collected zooplankton tows to compare prey in the water column over eelgrass and unvegetated sediment. We hypothesized that eelgrass invertebrates would migrate into the water column and that composition of tows over eelgrass canopy would have increased abundances of eelgrass-associated invertebrates.

The second objective was to quantify the contribution of eelgrass invertebrates to juvenile salmon diets. Due to the benthic nature of juvenile Chum Salmon feeding, we hypothesized that the majority of items found in juvenile Chum Salmon prey would also be found in eelgrass habitats. We complemented our diet analysis with $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope analysis of juvenile Chum Salmon and their potential diet sources (eelgrass invertebrates, zooplankton, and insects). The $\delta^{15}\text{N}$ signatures of consumers are 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 reflect those of their food source (DeNiro and Epstein 1978). We expected that eelgrass invertebrates would utilize eelgrass carbon (Ha et al. 2013) and reflect eelgrass stable isotope signatures, which are enriched in ^{13}C (Mittermayr et al. 2014) relative to terrestrial and marine sources (Doucet et al. 1996; Mittermayr et al. 2014). We expected that juvenile Chum Salmon would have $\delta^{13}\text{C}$ signatures that would reflect a diet of eelgrass invertebrates, thus indicating predation on eelgrass invertebrates.

METHODS

Study System

The Comox Estuary is on the east coast of Vancouver Island, British Columbia, within the Salish Sea (Figure 1). Eelgrass covers approximately 400 ha of the 1,470-ha estuary as a largely continuous patch of varying density. Much of the eelgrass is intertidal and accessible at low tide. The Puntledge River system feeding the estuary serves as rearing habitat for all five species of Pacific salmon (Trites et al. 1996) (for more details see the Supplement). The Puntledge Hatchery releases over 2 million juvenile Chum Salmon and over 1 million juvenile Chinook Salmon annually (Puntledge Hatchery, unpublished data), meaning most fish caught in this study were likely of hatchery origin. All sampling for this project was done in May, when juvenile Chum Salmon (Healey 1979) and Chinook Salmon (Healey 1991) outmigrate from the river and occur in peak numbers in the estuary.

Field Methods

Prey availability: epifaunal and infaunal eelgrass sampling.—Forty sampling points separated by at least 50 m were randomly generated over eelgrass in May 2015. Eelgrass was collected from a 0.0625-m² quadrat during low tide at each point. Quadrats were secured to the bottom and, if submerged, water depth was recorded. Shoots were harvested above the meristem and stored in 80% ethanol for analysis of eelgrass metrics and invertebrate abundance. Thirty additional intertidal samples were collected using the same methods in May 2016 during low tide. To investigate invertebrates in the sediment, an 8-cm-diameter, ~10-cm-deep sediment core was collected for a subsample of 29 of the eelgrass quadrats in 2015 and stored in 80% ethanol.

Prey availability: zooplankton and mobile invertebrate sampling methods.—To determine whether the presence of eelgrass would influence the composition of zooplankton and mobile invertebrates in the water column, tows were conducted at high tide in the Comox Estuary. Twelve samples, separated by a minimum of 50 m and chosen haphazardly, were conducted over eelgrass canopy and 12 over unvegetated sediment. Bottom depth and salinity and temperature at 1 m depth were measured at the beginning of each tow. The plankton tow net was made of 200- μm white Nitex mesh and had a diameter of 0.5 m, tapering to 10 cm at its base. A flowmeter suspended in the mouth of the net was used to correct for the volume of seawater sampled. The net was suspended just above the eelgrass canopy (tides were incoming during sampling, and net depth was adjusted accordingly but remained at approximately 1 m depth). The net was towed for 2 min at a speed of approximately 2.8 km/h (1.5 knots) while visually ensuring eelgrass was present or absent along the entire

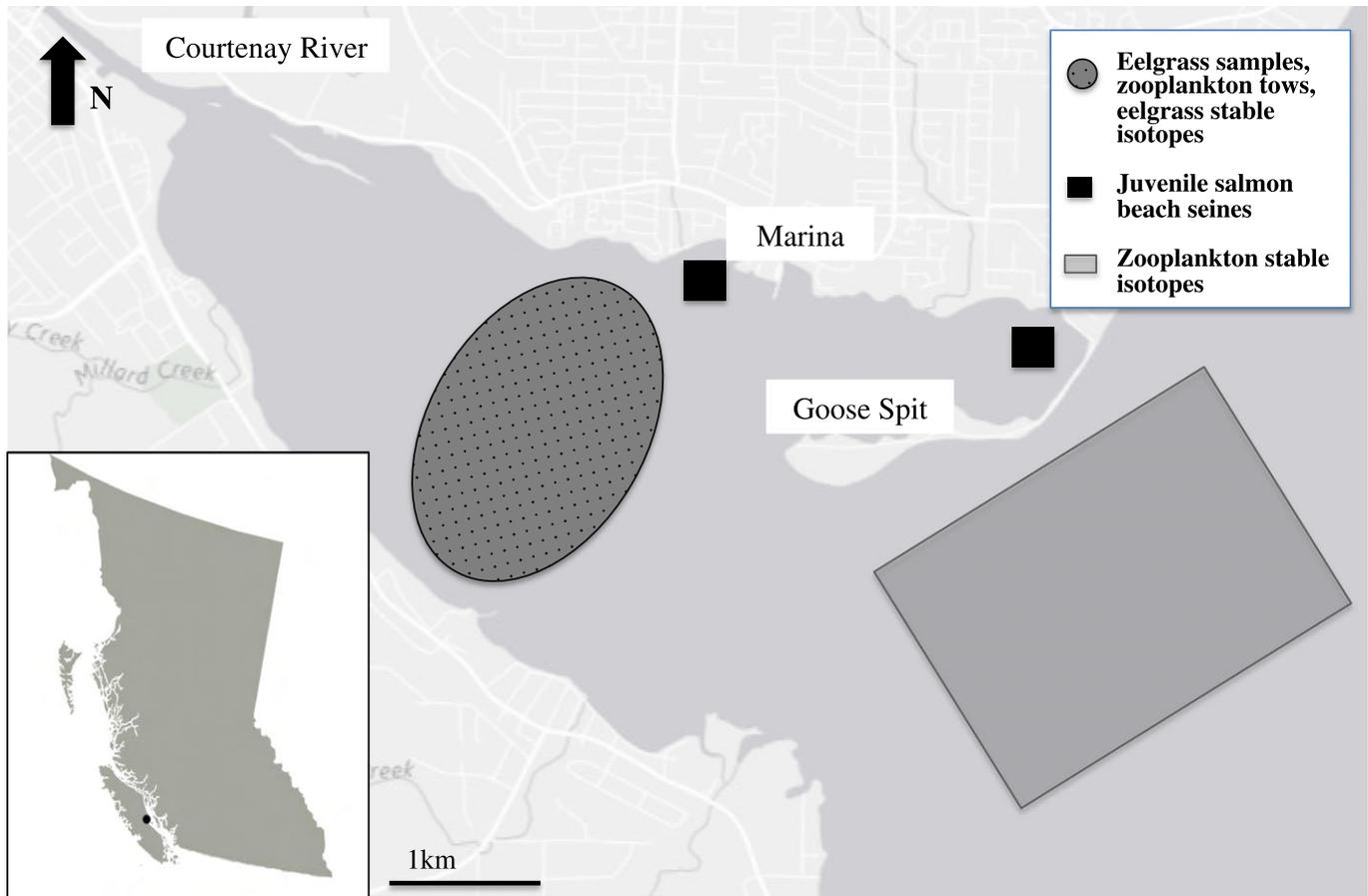


FIGURE 1. Sampling sites in the Comox Estuary, Vancouver Island, British Columbia, 2015 and 2016.

tow length. Invertebrates were collected and preserved in a solution of 4% formaldehyde for abundance analysis. Nighttime invertebrate sweeps (tows where water flow data were not recorded) were also performed to collect eelgrass invertebrates to account for the many invertebrates that migrate only at night (for more information see the Supplement).

Juvenile salmon diet: juvenile salmon sampling.— Juvenile salmon were collected for diet content analysis in two seine locations in the Comox Estuary to determine whether they were consuming eelgrass invertebrates (Figure 1). Over 2 d in 2015, 166 juvenile Chum Salmon were caught in a total of five tows during incoming tides using a 10 × 2-m beach seine with 3-mm mesh tapering to 1 m in height. Tow 4 was located west of Goose Spit, while all other tows were in Goose Spit (Figure 1). Juvenile Chum Salmon were euthanized with an overdose (300 mg/L) of tricaine methanesulfonate (MS222) in a seawater bath, weighed (± 0.1 g), and measured (FL, ± 0.1 cm). The spinal cord of each fish was broken to ensure mortality, and fish were placed on ice. Stomachs from all 166 fish were dissected and contents preserved in 80% ethanol for diet analysis.

The same methodology was used for 37 juvenile Chinook Salmon caught at Goose Spit in 2016 on an incoming tide. All salmon sampling was conducted with permits from Fisheries and Oceans Canada after approval of the Animal Care Committee of the University of Victoria.

Juvenile salmon diet: isotope sample collection.— At 10 of the most heavily vegetated eelgrass sampling points, eelgrass was harvested to collect epifaunal eelgrass invertebrates for stable isotope analysis (Figure 1). Zooplankton samples for stable isotope analysis were collected via two 5-min tows outside of the estuary with the zooplankton tow net (Figure 1). All samples, including juvenile salmon carcasses after the stomachs had been dissected, were frozen at -18°C . Literature values of -27.0 for $\delta^{13}\text{C}$ and 6.0 for $\delta^{15}\text{N}$ from black flies from the Miramachi River system in New Brunswick (Doucett et al. 1996) were used as estimates for terrestrial invertebrates, as sufficient weight requirements were not met from terrestrial samples collected. The values serve as a good proxy because the average $\delta^{13}\text{C}$ of terrestrial plants (-29.8) in Doucett et al. (1996) was similar to that in Howe Sound, British Columbia (-28.3) (Romanuk and Levings 2005), which,

like the Comox Estuary, is in the Salish Sea. 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.

Lab Processing

Prey availability: eelgrass epifaunal and infaunal prey invertebrates.—Ignoring the tallest 20%, three of the tallest eelgrass shoots from the remaining 80% were measured to the nearest millimeter to get the average eelgrass blade length (Short and Duarte 2001). Width was measured just above the meristem. Eelgrass was dried for 48 h at 60°C, then weighed to the nearest 0.0001 g to obtain biomass. Eelgrass invertebrates were picked from epiphytes scraped from eelgrass, and epiphytes were dried and weighed to the nearest 0.0001 g. Eelgrass invertebrates were separated into the lowest taxonomic group feasible. Similarly, invertebrates were identified and counted from each sediment core sample. Data were expressed as organism density/0.0625 m².

Prey availability: zooplankton and mobile invertebrates.—Large items such as medusae, larval fish, and large decapod shrimp (>1 mm), were removed from the zooplankton tow samples and enumerated. The samples were split using a Folsom splitter to ≥1,000 individuals per sample, and the remaining invertebrates were identified, counted, and expressed as organism density/m³.

Juvenile salmon diet: stomach contents.—Total weight and stomach lining weight from the esophageal opening of the stomach to the pylorus (Godin 1981) of the 166 juvenile Chum Salmon and 37 Juvenile Chinook Salmon were recorded to the nearest 0.0001 g. Gut invertebrates were sorted under a dissecting microscope, separated into the lowest taxonomic resolution feasible, and counted. 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).

Juvenile salmon diet: stable isotopes.—Samples for stable isotope analysis were comprised of dorsal muscle tissue dissected from a random sample of 15 of the 166 juvenile Chum Salmon, eelgrass invertebrates, and zooplankton invertebrates. Samples were rinsed in distilled water, dried for 48 hours at 50°C, ground to a homogeneous powder, and stored on silica gel desiccant in a sealed vial until packed and shipped for stable isotope analysis (Heck et al. 2008). Eelgrass invertebrates collected included amphipods, polychaete worms, copepods, and tanaids. Stable isotope analysis was conducted for individual polychaete worms and amphipods, which were pooled in groups of 5–10 to meet sample weight requirements; copepods and tanaids, however, did not meet sufficient sample weight requirements (~1 mg dry weight).

Zooplankton samples were rinsed of particulate organic matter, which left mostly calanoid copepods and crustacean nauplii. Zooplankton were analyzed in bulk; each dried sample weighed 3–5 mg and was composed of 100+ individuals.

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:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 1,000\%$$

where R_{sample} is the ratio of ¹³C:¹²C or ¹⁵N:¹⁴N in the sample, and R_{standard} is the ratio of ¹³C:¹²C in Pee Dee Belmenite or of ¹⁵N:¹⁴N in atmospheric nitrogen.

Data Analysis

Prey availability: effect of eelgrass density on abundance of epifaunal prey invertebrates.—Eelgrass shoot density/m² was categorized into low (0–49 shoots/m²), medium (50–99 shoots/m²), and high (100+ shoots/m²) for analysis (Webster et al. 1998), and average epifaunal and infaunal invertebrates were calculated. A generalized linear model (GLM) with a negative binomial distribution and a log link appropriate for count data skewed to low numbers (Bliss and Fisher 1953) was used to assess how eelgrass epifaunal prey invertebrate abundance was affected by eelgrass variables. Calcareous and large (>1 cm) items were excluded from analysis as they were not considered juvenile salmon diet items. Planktonic species such as crab megalopae, shrimp zoea, cyprid barnacles, decapod shrimp, euphasiid larvae, cladocerans, and ostracods were also excluded (Feller and Kaczynski 1975; Suthers and Rissik 2009), as this analysis focused on epifaunal species associated with eelgrass. Eelgrass density was chosen instead of other possible eelgrass metrics because it is a quick and inexpensive metric to measure (Fonseca et al. 1990) and is commonly used to assess eelgrass habitat damage or restoration success (e.g., Wright 2014; Yi et al. 2014; Eriander et al. 2016). The selection was justified by correlation analysis and comparison between models with all possible combinations of eelgrass covariates. For detailed information about prey availability see the Supplement.

Total epifaunal prey invertebrate abundance was modeled using eelgrass shoot density per sample (eelgrass density), epiphyte biomass per shoot (epiphyte biomass), year, and an interaction among epiphyte biomass and eelgrass shoot density. Continuous predictor variables were standardized to allow for a more direct comparison of the strength of the coefficients for each explanatory variable (Schielzeth 2010). A likelihood ratio test was performed to assess the significance

of including year in the model and to compare the fit of the negative binomial distribution to a Poisson distribution. Spatial autocorrelation in prey invertebrate abundance was assessed with Moran's *I* statistic (Moran 1950).

Prey availability: the effect of eelgrass density on the composition of epifaunal prey invertebrates.—A constrained analysis of principal coordinates (CAP) based on a Bray–Curtis distance matrix was used to visualize differences between epifaunal prey invertebrate composition across low, medium, and high eelgrass densities. Each invertebrate count was divided by the maximum count for that species to compute relative abundances of species, which allowed for rare and abundant species to be equally weighted (Jongman et al. 1995). Eelgrass density, epiphyte biomass, and year were included as explanatory variables. An ANOVA-like permutation test was performed to determine whether the CAP model was significant and to determine which explanatory variables were significant predictors of invertebrate composition. Variance partitioning was then used to determine how much variation in eelgrass epifaunal prey invertebrate composition was explained by each significant variable. Autocorrelation was assessed using a Mantel test with 1,000 permutations.

Juvenile salmon diet: stomach contents of juvenile salmon.—Species accumulation curves were used to assess whether juvenile salmon diets were adequately described (for detailed information see the Supplement). The proportion of each invertebrate group by count was assessed in juvenile Chum Salmon and juvenile Chinook Salmon diets using a stacked bar graph. The proportion of total abundance of eelgrass-

associated invertebrates, planktonic invertebrates, and terrestrial invertebrates in juvenile Chum Salmon and juvenile Chinook Salmon diets were calculated. To investigate the factors influencing the proportion of eelgrass-associated invertebrates in juvenile salmon diets, a CAP was performed on juvenile salmon diets using the following juvenile salmon metrics: FL, fish condition, gut fullness, tow number, and level of digestion as explanatory variables using the same procedure as per the epifaunal CAP (for selection and calculation of salmon metrics see the Supplement).

Juvenile salmon diet: stable isotopes.—Stable isotope analysis was used to estimate the contributions of eelgrass prey invertebrates, zooplankton, and terrestrial invertebrates to the diets of juvenile Chum salmon. Juvenile Chum Salmon data were lipid-corrected using the following equation from Post et al. (2007):

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

As stable isotope ratios undergo trophic enrichment, building up higher in the food chain, values of 0.4 $\delta^{13}\text{C}$ and 3.4 for $\delta^{15}\text{N}$, with SD values of 2, were assumed as trophic enrichment factors for juvenile Chum Salmon (Post 2002; Hertz et al. 2016). Without this step, the

MixSIAR Bayesian mixing model (Stock and Semmens 2013) used to estimate probability distributions of each diet source towards juvenile Chum Salmon would have overestimated the stable isotope signatures of the true diet of juvenile Chum Salmon. The analysis was performed using uninformative priors assuming each combination of diet items was equally likely (Stock and Semmens 2013). As this method favors a generalist diet, and additional diet information was available, the analysis was then redone using informative priors generated from our gut content analysis (0.8% for insects, 92.9% for eelgrass invertebrates, and 6.3% for zooplankton) constructed following Stock and Semmens (2013). All statistical analyses were conducted in R 3.2.1 (R Foundation for Statistical Computing, Vienna), using the vegan package (Oksanen et al. 2014). All CAP analyses were performed using the Cap-scale function in the vegan package (Oksanen et al. 2014).

RESULTS

Prey Availability: The Effect of Eelgrass Density on Abundance of Epifaunal Invertebrates

Items excluded from analysis because they were too calcareous or large for juvenile salmon to eat constituted 20.6% of the total abundance of epifaunal invertebrates. The excluded planktonic invertebrates represented 0.2% of the total abundance. Eelgrass blades hosted a total of 10 epifaunal prey invertebrate groups (Table 1), many of which were also found in lower abundances in infaunal samples. While epifaunal prey invertebrate counts were variable, harpacticoid copepods were the dominant invertebrate group by numerical abundance across all samples (Table 1; for more information on abundance see the Supplement).

The final model of epifaunal prey invertebrate abundance included eelgrass shoot density as the most important eelgrass metric to predict epifaunal prey invertebrate abundance (for more information see the Supplement), epiphyte biomass (g/shoot), year, and an interaction between eelgrass shoot density and epiphyte biomass (Table 2). A likelihood ratio test indicated the negative binomial model explained significantly more deviance than a Poisson model and was thus more appropriate ($\chi^2_5 = 31285.6$, $P < 0.0001$). There was no significant spatial autocorrelation in epifaunal prey invertebrate abundance across eelgrass quadrats taken ≥ 50 m apart (Moran's *I*: $z = 0.940$, $P = 0.347$). When epiphyte biomass was held at its mean (52.86 mg), every unit of SD in eelgrass shoot density caused epifaunal prey invertebrate abundance to increase with a coefficient of 0.146 on the log scale (Table 2; Figure 2). Year was a significant predictor of epifaunal invertebrate abundance ($\chi^2_{71,72} = 6.470$, $P = 0.039$) (Table 2). Epifaunal invertebrate abundance

was also positively influenced by epiphyte biomass (Table 2).

Prey Availability: Effect of Eelgrass Density on the Composition of Epifaunal Invertebrates

There was no significant autocorrelation in epifaunal prey invertebrate communities at a distance of 50 m based on a Mantel test with 1,000 permutations ($P = 0.171$). The CAP of epifaunal prey invertebrate abundance was significant ($F_{3, 64} = 4.687$, $P = 0.001$), explaining 17.6% of the variation in the epifaunal prey invertebrate community (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 (Figure 3). 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% of the variance,

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 quadrats separated from low- and medium-density eelgrass quadrats along the first axis (Figure 3). Most epifaunal prey invertebrate groups were positively associated with eelgrass density and epiphyte biomass in epifaunal samples (Figure 3).

Prey Availability: Effect of Eelgrass Presence on Invertebrate Availability in the Water Column

Invertebrates associated with eelgrass, such as polychaete worms and crustaceans such as harpacticoid copepods and amphipods, were not abundant in the zooplankton during the day in 2015 (Table 3). These invertebrates were also not seen in invertebrate sweeps during the evening in 2016. The CAP of zooplankton communities was significant based on an ANOVA-

TABLE 1. Average and SD of eelgrass epifaunal prey invertebrate counts in low-, medium-, and high-density eelgrass quadrats in the Comox Estuary, British Columbia, May 2015 and May 2016. Averages are rounded to the nearest whole number.

| Taxon | Low (0–49 shoots/m ²) | | Medium (50–99 shoots/m ²) | | High (100+ shoots/m ²) | |
|--------------------------------|-----------------------------------|-------------------|---------------------------------------|-------------------|------------------------------------|-------------------|
| | 2015 average (SD) | 2016 average (SD) | 2015 average (SD) | 2016 average (SD) | 2015 average (SD) | 2016 average (SD) |
| Annelida | | | | | | |
| Small polychaete worms (<5 cm) | 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) | 1,179 (1,196.2) |
| 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 (<1 cm) | 0 (0) | 0 (0) | 0 (0) | 0 (0.3) | 0 (0.3) | 1 (2.1) |
| 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 2. Summary of the negative binomial model of epifaunal prey invertebrate abundance in eelgrass quadrats from the Comox Estuary, British Columbia, May 2015 and May 2016. (Null deviance: 126.53 on 74 df, residual deviance: 90.85 on 70 df, $2 \times \log$ -likelihood: $-1,037.99$).

| Model parameter | Estimate | SE | Z-value | P-value |
|---|----------|---------|---------|---------|
| Intercept | 4.732 | 0.239 | 19.774 | <0.0001 |
| Year | 0.708 | 0.265 | 2.669 | 0.007 |
| Shoot density (shoots/0.0625 m ²) | 0.146 | 0.029 | 4.921 | <0.0001 |
| Epiphyte biomass (g/shoot) | 0.008 | 0.003 | 2.854 | 0.0043 |
| Shoot density \times epiphyte biomass | <0.0001 | <0.0001 | 0.001 | 0.522 |

like permutation test that used 999 permutations ($F_{5, 18} = 1.526$, $P = 0.013$) (Figure 4) and indicated a total of 29.8% of the total variation in species composition was constrained by the explanatory variables. Tow type was the only significant variable ($F_{1, 18} = 2.741$, $P = 0.003$), while salinity ($F_{1, 18} = 1.140$), temperature ($F_{1, 18} = 0.148$), volume of seawater sampled ($F_{1, 18} = 1.200$), and bottom depth ($F_{1, 18} = 0.917$) were not (all $P > 0.1$). Alone, tow type explained 9.9% of the total constrained variation; however, vegetated and unvegetated tows did not cluster together on the graph, indicating that the presence of eelgrass did not greatly affect tow invertebrate composition (Figure 4).

Juvenile Salmon Diet: Stomach Contents of Juvenile Salmon

The number of diet species increased with sample size, as expected, but samples of 166 juvenile Chum Salmon (FL, 32–75 mm) and 37 juvenile Chinook Salmon (FL, 60–85 mm) were adequate to describe juvenile salmon diet for the purposes of this study. A variety of prey items were observed in juvenile Chum Salmon diets, including zooplankton species such as crustacean zoea, decapod shrimp, crab megalopae, euphasiid larvae, ostracods, cladocerans, nauplii, cyprids, and hyperid amphipods, terrestrial species including spiders and dipterans, and eelgrass-associated species, such as gammarid amphipods, harpacticoid copepods, polychaete worms, cumaceans, and snails (Figure 5). Juvenile Chinook Salmon had similar diet items (Figure 5).

Harpacticoid copepods dominated invertebrate counts in diets of both salmon species (Figure 4). Polychaete worms and cladocerans were the next most important groups for juvenile Chum Salmon numerically, while decapod shrimp and crab megalopae were the next most

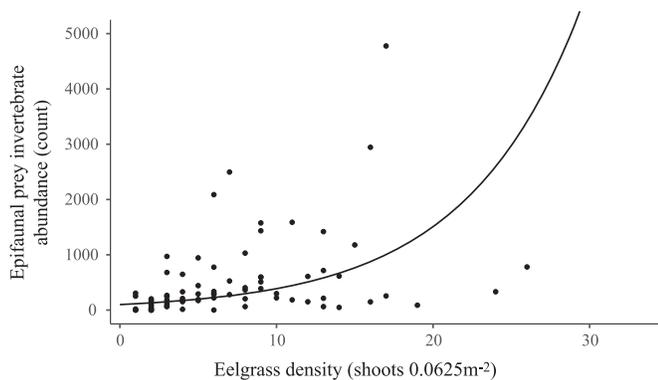


FIGURE 2. Predicted values of negative binomial model of epifaunal prey invertebrate abundance as a function of eelgrass density in (shoots/0.0625 m²) in eelgrass quadrats collected in May 2015 and May 2016 in the Comox Estuary, British Columbia. Year is held at its centered value and epiphyte biomass (g/shoot) at its mean.

important for juvenile Chinook Salmon numerically (Figure 4). Eelgrass-associated species made up about 93% and 83% of juvenile Chum Salmon and Chinook Salmon diets, respectively. Planktonic species made up about 6% and 13% of juvenile Chum Salmon and juvenile Chinook Salmon diets, respectively, followed by terrestrial insects, which made up approximately 1% and 4% of those diets, respectively. For detailed information on the stomach contents of juvenile salmon see the Supplement.

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 6). Salmon metrics, including juvenile salmon FL, 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 6). 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 with 999 permutations but only explained 0.04% and 0.02% of the variation, respectively (Figure 6).

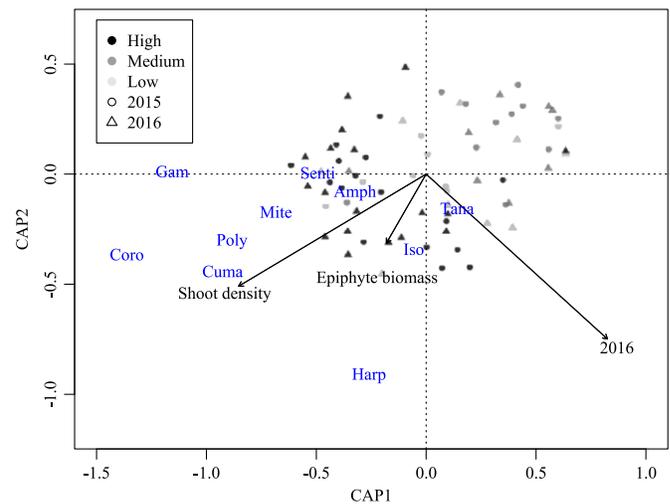


FIGURE 3. Constrained analysis of principal coordinates (CAP) of epifaunal prey invertebrate communities across low- (0–49 shoots/m²), medium- (50–99 shoots/m²), and high-density (100+ shoots/m²) eelgrass collected from 0.0625-m² quadrats in May 2015 and May 2016 from the Comox Estuary, British Columbia. Shoot density (shoots/0.0625 m²), epiphyte biomass (g/shoot²), and year are explanatory variables, and the length of the arrows indicate the strength of each predictor. Each point on the graph represents the epifaunal prey invertebrates found in a single quadrat, in which density categories are coded as different shadings 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.

Juvenile Salmon Diet: Stable Isotope Analysis

On an isotopic biplot of $\delta^{13}\text{C}$ against $\delta^{15}\text{N}$, juvenile Chum Salmon stable isotope signatures ($\delta^{13}\text{C}$: -14.79 ± 1.85 [mean \pm SD], $\delta^{15}\text{N}$: 12.43 ± 0.72 , $n = 15$) most closely overlapped those of eelgrass prey invertebrates ($\delta^{13}\text{C}$: -14.65 ± 1.39 , $\delta^{15}\text{N}$: 8.43 ± 2.55 , $n = 6$) compared with terrestrial insects, which were the most depleted in $\delta^{13}\text{C}$ ($\delta^{13}\text{C}$: -27.00 ± 2 , $\delta^{15}\text{N}$: 6 ± 2 , $n = 4$), followed by zooplankton ($\delta^{13}\text{C}$: -18.82 ± 0.3 , $\delta^{15}\text{N}$: 8.63 ± 0.38 , $n = 10$) (Figure 7). Using informative priors, eelgrass invertebrates made up the highest mean percent of juvenile Chum Salmon diet ($79.8 \pm 10.2\%$), with Bayesian credible intervals ranging from 7.9% to 95.2% (Table 4). 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 4). Results were similar using informative priors, strongly

favoring eelgrass invertebrates as the top diet source (Table 4).

DISCUSSION

Disentangling the relationship between the early life history of juvenile salmon and eelgrass habitats may further our understanding of the impacts of eelgrass loss. We focused on the role of eelgrass habitat in creating foraging opportunities for juvenile Chum Salmon and juvenile Chinook Salmon. Eelgrass hosted a variety of prey invertebrates consumed by juvenile salmon. The most numerically abundant taxon in juvenile Chum Salmon and juvenile Chinook Salmon diets was harpacticoid copepods, which we only found in high abundance in eelgrass habitat throughout the Comox Estuary, compared with those in sediments and zooplankton. We complemented these findings with stable isotope analysis, revealing that

TABLE 3. Temperature, salinity, and bottom depth of zooplankton tows, and average, SD, and total density/m³ (Total density) of zooplankton caught in inner-estuary tows over eelgrass and unvegetated sediment in Comox Estuary, British Columbia, May 2015. Averages are rounded to the nearest whole number.

| Measure/taxon | Unvegetated estuary | | Vegetated estuary | |
|--------------------------------|---------------------|---------------|-------------------|---------------|
| | Average (SD) | Total density | Average (SD) | Total density |
| Temperature (°C) ^a | 15.0 (1.8) | | 14.3 (0.7) | |
| Salinity (‰) ^a | 29.5 (0.6) | | 29.6 (0.9) | |
| Depth (m) | 2.1 (0.9) | | 2.3 (0.6) | |
| Chordata | | | | |
| Juvenile fish | 0 (0.5) | 3 | 6 (8.8) | 73 |
| Annelida | | | | |
| Small polychaete worms (<5 cm) | 3 (7.1) | 41 | 13 (28.8) | 155 |
| Mollusca | | | | |
| Sea snails | 0 (0) | 0 | 1 | 8 |
| Crustacea | | | | |
| Harpacticoid copepods | 4 (6.4) | 45 | 27 (56.7) | 320 |
| Calanoid copepods | 238 (241.9) | 2,853 | 1,876 (1,445.7) | 22,512 |
| Barnacle cyprids | 51 (55.7) | 613 | 62 (111.7) | 746 |
| Shrimp zoea | 8 (17.9) | 93 | 26 (45.2) | 315 |
| Crab zoea | 5 (5.8) | 57 | 22 (62.5) | 270 |
| Crab megalops | 0 (0) | 0 | 0 (0) | 1 |
| Euphasiid zoea | 1 (1) | 9 | 11 (27.4) | 130 |
| Cladocerans | 292 (396.0) | 3,505 | 1,534 (2,564.8) | 18,413 |
| Amphipoda | | | | |
| Malacostraca | | | | |
| Small isopod (<1 cm) | 0 | 0 | 1 (2.7) | 15 |
| Hyperid amphipods | 0 (1.4) | 5 | 0 (0) | 0 |
| Senticaudata | | | | |
| Gammarid amphipods | 0 (0) | 1 | 0 (0) | 0 |
| Insecta | | | | |
| Dipterans | 0 (0) | 1 | 0 (1) | 1 |

^aSalinity and temperature measured at 1 m depth.

the diets of juvenile Chum Salmon were comprised mainly of eelgrass-associated invertebrates (though these species may also be found in other locations in the estuary that were not sampled). The abundance of harpacticoid copepods and other eelgrass-associated prey invertebrates increased linearly with higher eelgrass densities, suggesting that habitat features like eelgrass increase prey abundance in nearshore 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 et al. 2010).

Prey Availability: Epifaunal and Infaunal Eelgrass Invertebrates

The invertebrate species found in the Comox Estuary were similar to those found in the study by Knight et al. (2015) in Tsawwassen, British Columbia, also in the Salish Sea. The observation that epifaunal invertebrates increase with eelgrass density is consistent with our hypothesis and supported research from other locations (Orth et al. 1984; Fonseca et al. 1990; Boström et al. 2006; Hosack et al. 2006; Möller et al. 2014; Hovel et al. 2016). Epiphyte load also positively influenced the total abundance of epifaunal prey invertebrates (Table 2), epiphytes being a major source of productivity and a trophic resource for seagrass invertebrates (Bologna and Heck 1999), though these

effects are not seen in all eelgrass habitats (e.g., Knight et al. 2015). Because eelgrass invertebrates exhibit top-down control on epiphytes (Whalen et al. 2013; Michel et al. 2015) results can differ, and thus invertebrate abundance may not always follow a simple linear relationship with epiphyte biomass. Year was also a significant contributor to epiphyte prey invertebrate abundance in the negative binomial model indicating interannual variation in this relationship and supporting the hypothesis that eelgrass invertebrates exhibit yearly and seasonal fluctuations (Heck and Orth 1980).

Because invertebrates display species-specific responses to seagrass structure (Schneider and Mann 1991), we hypothesized that as eelgrass density decreased from dense to sparse, the community composition would transition from eelgrass-associated species to sediment-associated species. The results from the CAP model do not support research finding compositional changes in eelgrass invertebrate communities in response to other eelgrass metrics (e.g., Sirota and Hovel 2006; Moore and Hovel 2010; Sutherland et al. 2013), because greater than 80% of the variation in epifaunal prey invertebrate communities remained unexplained by eelgrass variables (Figure 6). Our results instead suggest that eelgrass prey community composition is largely similar across a gradient of eelgrass density. This similarity is important to consider when assessing the value of eelgrass habitat. Eelgrass beds interspersed along the Howe Sound in the 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 any sparse eelgrass may host similar invertebrate communities that are found in the dense beds.

Prey Availability: Zooplankton Tows

Our hypothesis that eelgrass-associated prey drifted far from eelgrass shoots into the water column was not supported by the data based on the relative absence of eelgrass invertebrates in tows during the day in 2015 and in invertebrate sweeps during the day and evening in 2016. This conclusion was reinforced by the fact that tows conducted in eelgrass canopy and tows in unvegetated sediment overlapped in the CAP, indicating that the presence of eelgrass did not influence invertebrate composition in the water column (Figure 4). Invertebrates such as isopods and amphipods are highly mobile (Arponen and Boström 2012). Instead of migrating through the water column, however, mobile invertebrates in the Comox Estuary may migrate closer to the sediment bottom or between the leaves (Mascart et al. 2015). Our results suggest that the presence of eelgrass does not influence prey availability directly above eelgrass habitat. However, given that some invertebrates such as harpacticoid copepods are found only within the first 20 cm from the sediment bottom in

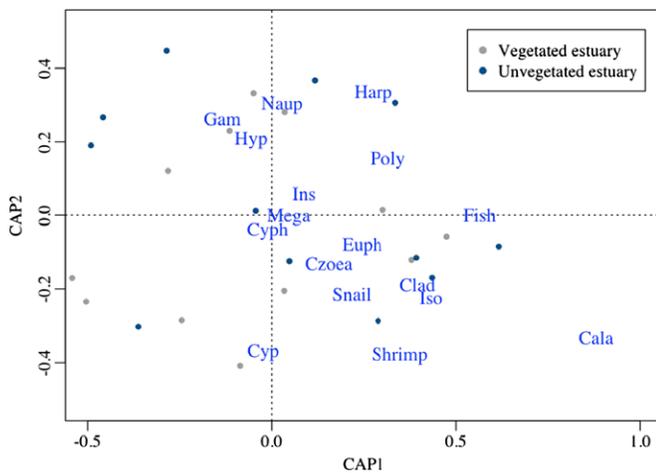


FIGURE 4. Constrained analysis of principal coordinates (CAP) of zooplankton communities from tows over eelgrass in vegetated and unvegetated estuary in the Comox Estuary, British Columbia, May 2015. Tow type (vegetated and unvegetated estuary) is the explanatory variable and is coded as gray or blue, respectively, and each point on the graph represents the invertebrates found in a single tow. Species are abbreviated for display purposes as follows: 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, Czoa = crab zoea, Shrimp = shrimp zoea, Fish = larval fish, Cala = calanoid copepods, Clad = clacoderans, Euph = euphasiid zoea, Cyph = cyphanaules larvae.

eelgrass (D'Amours 1988), there is still the potential that eelgrass influences the presence of invertebrates in the water column directly above the sediment bottom.

Juvenile Salmon Diet: Stomach Contents

Terrestrial insects were present in the diets of both salmon species; and dipterans occurred in high frequencies in juvenile Chum Salmon diets and ants were present in many juvenile Chinook Salmon diets (for detailed information on the stomach contents of juvenile salmon see the Supplement). Insects are established resources for juvenile Chum Salmon (Romanuk and Levings 2005; Sturdevant et al. 2012; Dumbauld et al. 2015) and juvenile Chinook Salmon (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 (~89%) and polychaete worms (~3.5%) by count. Invertebrates such as these made up over 90% of juvenile Chum Salmon diet by count. This harpacticoid-dominated diet is consistent with juvenile Chum Salmon (mean FL, 42 mm) in the Nanaimo Estuary (Healey 1979) and within Puget Sound (Feller and Kaczynski 1975 [FL, 30–59 mm]; Simenstad and Cordell 2000 [FL, 40–50 mm]), locations where eelgrass is present. Juvenile Chinook Salmon prey

were more planktonic than were prey in juvenile Chum Salmon diets; planktonic species, mainly decapod shrimp and crab megalopae, made up 30% of juvenile Chinook Salmon diets by count. These diet differences were reflected in the CAP, in which individuals of the same species clustered closer together. Juvenile salmon metrics, however, did not strongly influence the proportion of eelgrass invertebrates in the diets. Given that juvenile salmon feed opportunistically and undergo ontogenetic diet shifts (Dumbauld et al. 2015), we expected that salmon metrics, such as length, would influence the diets. However, the length ranges of the salmon we measured and our sample sizes may not have been large enough to detect such ontogenetic diet shifts.

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). While we did not sample all habitat features in the estuary due to time constraints, *Harpacticus uniremus*, a common copepod targeted by juvenile Chum Salmon (Healey 1979; Sibert 1979; Webb 1991), is strongly associated with eelgrass beds (Webb and Parsons 1992; Hosack et al. 2006). Copepods are also more abundant in eelgrass blades than they are in sediment even within an eelgrass

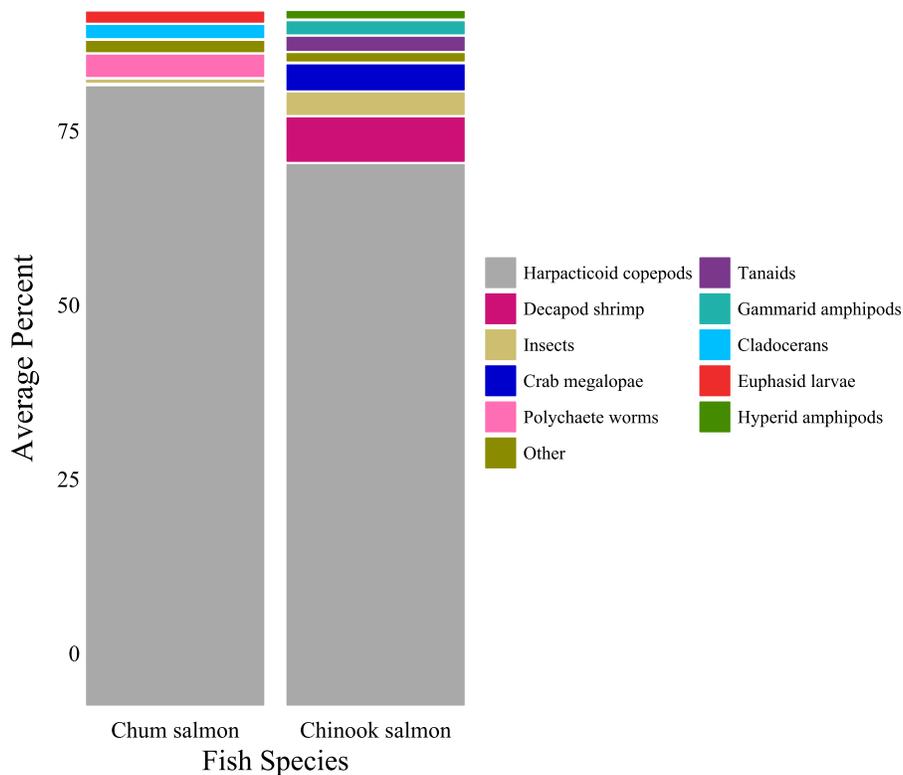


FIGURE 5. Average percent of invertebrate counts in diets of juvenile Chum Salmon caught in 2015 and juvenile Chinook Salmon caught in 2016 in the Comox Estuary, British Columbia.

bed (Webb and Parsons 1992), and in some cases, copepod assemblages targeted by juvenile Chum Salmon are present only in eelgrass and missing from other habitats such as sand or mud flats (Simenstad et al. 1988). In the Comox Estuary, eelgrass samples were the only samples containing an abundance of harpacticoid copepods compared with sediment and zooplankton samples, suggesting that eelgrass provided juvenile salmon with access to the abundance of copepods in their diets. While eelgrass habitat may be a notable supplier of harpacticoid copepods for juvenile salmon, it is important to note that harpacticoid copepods have relatively low individual biomass compared with zooplankton such as shrimp zoea. Furthermore, juvenile salmon use a variety of habitats and may not always favor harpacticoid copepods. For example, marsh habitat in the Comox Estuary may supply more prey for salmon favoring insects than do eelgrass beds (Duffy et al. 2010).

Juvenile Salmon Diet: Stable Isotope Analysis

Stable isotope analysis strengthened our conclusion that juvenile Chum Salmon in the Comox Estuary foraged on eelgrass-associated prey. Using the MixSIAR model, we estimated the most probable value for the percent of

eelgrass invertebrates in the diets of juvenile Chum salmon was ~80%, and possible contributions ranged from ~6% to 94%. Juvenile Chum salmon in our study had similar stable isotope signatures to those sampled in Howe Sound ($\delta^{13}\text{C}$: -15.15 ± 1.82 [mean \pm SD] compared with -19.35 ± 1.07 , and $\delta^{15}\text{N}$: 12.53 ± 0.73 compared with 12.9 ± 1.16 , respectively) (Romanuk and Levings 2005). While we acknowledge the potential that our fish were of hatchery origin, their stable isotope signatures are not reflective of their hatchery feed diet, which is composed of fish meal and oils from forage fish (Puntledge Hatchery, unpublished data), given their relatively low $\delta^{15}\text{N}$ signature. For example, the average $\delta^{15}\text{N}$ signature of Pacific Herring *Clupea pallasii*, from which hatchery meal could be sourced, is $\delta^{15}\text{N}$ 12.9 (Hertz et al. 2016). The $\delta^{15}\text{N}$ signature of juvenile Chum Salmon was 12.4, representing a lower trophic level than feed used in the fish hatchery. While we did not collect sufficient samples of nonplanktonic invertebrates from outside eelgrass habitat to distinguish eelgrass invertebrates from others, the low counts of invertebrates in sediment samples indicate that many invertebrates eaten by juvenile salmon in the estuary likely came from eelgrass. In addition, eelgrass and epiphyte carbon can be deposited throughout an estuary, so any nonplanktonic invertebrates consumed by juvenile salmon in the estuary are likely supplemented by carbon associated with eelgrass beds (Ha et al. 2013). Due to time constraints in

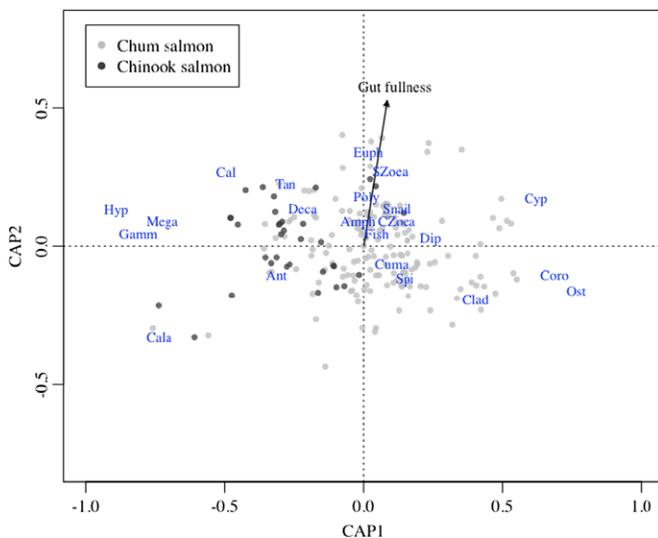


FIGURE 6. Constrained analysis of principal coordinates (CAP) of juvenile salmon diets in the Comox Estuary, British Columbia, May 2015. Salmon species is the explanatory variable coded as different shadings, with each point on the graph representing the invertebrates found in an individual diet. Species are abbreviated for display purposes as follows: Amph = other amphipods, Poly = polychaete worms, Harp = harpacticoid copepods, Hyp = hyperiid amphipods, Cyp = barnacle cyprids, Mega = crab megalops, Snail = sea snails, CZoea = crab zoea, SZoea = shrimp zoea, Fish = larval fish, Cala = calanoid copepods, Clad = clacoderans, Euph = euphasiid zoea, Ant = terrestrial ants, Coro = corophid amphipods, Dip = dipteran flies, Deca = decapod shrimp, Ost = ostracods, Spi = spiders, Tan = tanaids.

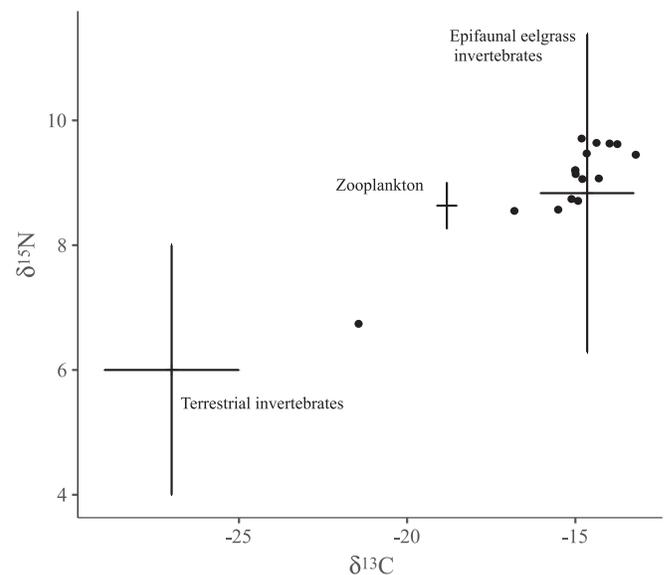


FIGURE 7. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ stable isotope signatures of juvenile Chum Salmon (solid dots) ($n = 15$) and three potential carbon sources (epifaunal eelgrass prey invertebrates [$n = 6$], zooplankton [$n = 10$], and terrestrial invertebrates [$n = 4$]) from the Comox Estuary, 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 $\delta^{15}\text{N}$. Error bars represent \pm SD.

TABLE 4. Mean, SD, and Bayesian credible intervals for estimated diet proportions of food sources to the diet of juvenile Chum Salmon in the 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) | 75.8 (8.0) | 7.9–95.2 | 56.6–88.7 |
| Insects | 4.2 (3.4) | 14.7 (5.3) | 0.1–12.6 | 5.4–27.1 |
| Zooplankton | 16.9 (11.0) | 9.5 (7.0) | 0.7–41.3 | 0.2–25.6 |

2016, the same analysis was not conducted for juvenile Chinook Salmon, though this may be an interesting avenue of future research to supplement these findings.

Management Implications

Juvenile salmon use nearshore habitat extensively during the early marine period (Duffy et al. 2005), a critical time for salmon growth, as larger, faster-growing fish have increased probabilities of surviving to adulthood (Beamish et al. 2003; Duffy and Beauchamp 2011). While mechanisms that determine the growth of fish in estuarine environments are poorly understood, the capacity of these habitats to provide prey is likely an important component of salmon success (Simenstad and Cordell 2000). In our study, juvenile Chum Salmon and juvenile Chinook Salmon diets were dominated by harpacticoid copepods by count, which were found in abundance in epifaunal samples taken in eelgrass. Juvenile Chum Salmon also displayed stable isotope signatures that imply the use of eelgrass habitat for foraging opportunities.

Eelgrass habitat is a valuable component of the ecosystem continuum through which salmon migrate, and continued efforts should be made to adhere to the “No Net Loss” policy for eelgrass in British Columbia, including routine monitoring for habitat compensation. Such attention is especially critical given that invertebrate densities are significantly decreased below a certain threshold of eelgrass density (Reed and Hovel 2006). As juvenile salmon appear to be critical species benefitting from maintaining the integrity of eelgrass habitat, it is crucial to continue mapping and monitoring eelgrass habitat and record the availability of this habitat for juvenile salmon. These baselines also allow managers to track responses of eelgrass to anthropogenic pressures. It is also important to keep in mind that anadromous Pacific salmon feed opportunistically, and their diets vary temporally, spatially, and between species (Bollens et al. 2010; Hertz et al. 2015). Eelgrass and other structured estuarine habitats such as salt marsh and oyster beds may not provide food sources for juvenile salmon that have already shifted their diet niches to planktonic species (Bollens et al. 2010). Access to productive foraging grounds across the ecological landscape through which

juvenile salmon migrate, not just eelgrass habitat, may impart increased survival chances.

ACKNOWLEDGMENTS

This is Publication Number 16 from the Salish Sea Marine Survival Project (marinesurvivalproject.com). We thank the Pacific Salmon Foundation for funding this project, and also would like to acknowledge the Canadian Foundation for Innovation, the British Columbia Knowledge Development Fund, and Natural Sciences and Engineering Research Council Discovery Grants for equipment and lab technician support. We thank the numerous people who assisted with collection and processing samples for this project, as well as Project Watershed and the Sea-Change Society for support. Dr. Moira Galbraith assisted with zooplankton and stomach contents identification for this project. We thank Dr. Julia Baum and Dr. Natalie Ban for helpful suggestions on an earlier draft, as well as the reviewers whose insight greatly improved this publication. We would also like to thank Geoffrey Osgood for statistical guidance. There is no conflict of interest declared in this article.

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the supporting information tab for this article.