Effects of Seasonal Availability and Energy Content of Key Prey on Growth Performance during Critical Periods for Juvenile Chinook Salmon

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INTRODUCTION

The marine survival of juvenile Chinook salmon in Puget Sound is strongly influenced by size-selective mortality, which for hatchery-origin juveniles, has been highly correlated to the body mass achieved while feeding in open-water habitats during a critical growth period in mid-June through July (Duffy and Beauchamp 2011). Early marine growth is food-limited, and variability in growth, feeding, and survival can largely be accounted for by the variable contribution of larval crab to the energy budget of juvenile Chinook during the critical growth period during June-July (Beauchamp and Duffy 2011, Duffy and Beauchamp 2011, Gamble 2016). Little is known about how the spatial-temporal availability of larval crab relates to feeding and growth of juvenile Chinook salmon, but this knowledge could become a fundamental element in the recovery of these stocks by identifying important functional components for habitat restoration and informing management of water quality and quantity.

How juvenile salmonids initially allocate the energy they consume into either somatic growth (size) or energy stores strongly influences how fast they grow, how much they need to eat to achieve a given growth rate, and thus change their vulnerability to size-selective predation or overwinter mortality (Biro et al. 2005, 2006). The energy densities of juvenile Chinook salmon and their primary prey, and changes in energy density through time or among developmental stages of consumers or their prey are key uncertainties when estimating the feeding rate and consumption demand (Breck 2008) required to achieve the size that ultimately influences marine survival.

Bioenergetics modeling provides a useful analytical framework for diagnosing the primary factors that affect growth or predation rates, because the model simulations account for daily dynamics in thermal regime, diet and the associated energy density of prey, and changes in the body mass and associated energy density of the consumer. Model predictions of growth, feeding rate, and consumption demand are sensitive to dynamics in energy density of both salmon and their primary prey. Therefore, understanding the temporal and spatial dynamics of larval crab availability and their energy contribution to juvenile salmon growth has become a key uncertainty in developing a mechanistic understanding of what limits marine survival of juvenile Chinook salmon and potentially other salmon and forage fish species in the Salish Sea. Based on temporal and habitat-specific changes in diet composition, we identified which taxa, sizes, and developmental stages of key prey (i.e., larval crab) represented the edible fraction of the forage base and then summarized the temporal-spatial patterns of ambient prey densities among regions and through time for juvenile Chinook salmon feeding in epi-pelagic habitats during 2014-2015. We then experimentally examined how variable densities of crab larvae affected feeding rates of juvenile Chinook salmon. Lastly, we examined how energy densities of juvenile Chinook salmon and larval crab changed through time and influenced Chinook growth during their critical growth period.

The objectives of this study were to: **1)** examine the spatial-temporal availability of the edible size fraction of larval crab during the critical growth period for juvenile salmon; **2)** determine how larval crab densities affected feeding rates of juvenile Chinook salmon using functional response experiments (e.g., Moss and Beauchamp 2007; Haskell et al. 2017); **3)** determine the change (if any) in energy density (% dry mass and J/g wet mass from bomb calorimetry) of crab zoea and megalops as a function of larval body size, time (month), and, if possible, region; **4)** measure the energy density of juvenile Chinook salmon as functions of body mass and month.

METHODS

Spatial-Temporal Variability in Availability of Edible Crab Larvae

We identified the species of larval crab and measured the sizes larval crab in diets of Chinook salmon sampled in offshore habitats during 2014 and 2015. The proportional contribution of these prey in the diet and sizes were compared to the analogous prey densities and sizes from concurrent sampling with 0-30 m deep oblique Bongo net tows. We measured the carapace lengths (CL, mm) of zoea and megalops following methods of Hirota and Fukuda (1985). After the predominant larval crab species were identified and measured from the diet samples, the larval crab densities from the Bongo net data were re-analyzed in terms of the spatial-temporal patterns in exploitable species and sizes of crab larvae that were available to juvenile Chinook salmon in various regions of Puget Sound during the critical June-July growth period offshore.

Spatial-temporal patterns in numerical and biomass density of exploitable zooplankton were compared to corresponding estimates of diet composition, feeding and growth rates for juvenile Chinook to determine how feeding and growth was influenced by variability in the biomass of edible size fractions of larval crab. The observed patterns in prey availability were be applied to functional response curves (see section below) and linked to bioenergetics simulations to evaluate the sensitivity of Chinook feeding and growth to the range of variability in prey availability experienced during critical growth periods.

Functional Response Experiments-Functional response experiments provide valuable insights into how feeding rates by individual consumers are affected by changes in ambient densities of key prey and can be designed to evaluate how feeding capabilities change with body size of consumer, prey or both (Koski and Johnson 2002; Moss and Beauchamp 2007; Haskell et al. 2017). We examined the feeding rate (numbers of prey eaten/minute) of juvenile Chinook salmon as a function of density of crab megalops, and repeated a limited number of trials using Z5 zoea. We used red rock crab (Cancer as prey in these experiments, because this was the most common taxa and size of megalopae (mean carapace length CL = 1.9 mm (SD = 0.4 mm)) observed in diets of juvenile Chinook salmon during the critical June-July growth period in epi-pelagic habitats of Puget Sound during 2014 and 2015 (see section below).

Experimental prey densities (1-500 prey/m^3) were employed to bracket the range of densities observed in oblique Bongo net tows (over 0-30-m depths) across regions during the twice-monthly daylight sampling periods (April-August) conducted in Puget Sound during 2014 and 2015 (J. Keister and H. Schultz, unpublished data). In reality, the *in situ* densities for the *edible size fraction of larval crab* (>1.2 mm) ranged 0-12 megalops/m^3 during the June-July critical growth period. However, the higher experimental prey densities allowed us to test whether foraging salmon required higher-density patches of prey to achieve the feeding rates needed to support rapid growth during the critical period. Such high-density patches would not be detectable by the Bongo net samples used to estimate zooplankton densities available to foraging salmon during daylight. The Bongo nets (and foraging salmon) could be sampling expanses of low-density prey, punctuated by infrequent high-density patches. We used two size categories of Chinook salmon to emulate the modal size ranges of juveniles observed along shoreline (nearshore) habitats (80-100 mm FL) and in epi-pelagic (offshore) habitats (120-140 mm FL) during the critical growth period in June-July (Duffy et al. 2010, Beauchamp and Duffy 2011; Gamble et al. 2018).

Juvenile Chinook were obtained from the Garrison Springs Hatchery in Tacoma operated by Washington Department of Fish and Wildlife (WDFW Transport Permit #7757), then held at a mean temperature of 11.5 ^{o}C (SD = 0.5^{o}C, range = 10-13 ^{o}C), fed to satiation daily with hatchery pellets using automatic feeders, supplemented with larval crab to help acclimate the juvenile Chinook to feeding on natural prey. Larval crab (Z5 zoea and megalopae, *Cancer sp*) were collected repeatedly as needed using primarily aquarium dip light from docks in the city marina in Port Townsend, WA. The Z5 zoea and megalopae of Red Rock Crab (*Cancer productus*) were sorted, measured for carapace length (CL) following methods of Hirota and Fukuda (1985), and counted to achieve the densities required for each feeding trial.

Since juvenile salmon feed during daylight in epi-pelagic waters, the feeding experiments were conducted under ambient temperatures (mean = 11.5 ^{o}C, SD = 0.5^{o}C, range = 10-13 ^{o}C) and daylight conditions in indoor lab facilities (100-300 Lux). These conditions approximated the ambient conditions experienced at depth during the critical growth period in Puget Sound and spanned ranges where feeding rates were relatively insensitive to modest incremental changes in temperature (on metabolic consumption demand; Beauchamp 2009) and light-dependent visual foraging capabilities (Hansen et al. 2013).

The feeding trials during 2017 were handicapped by juvenile Chinook that were too small to emulate the target consumer group, and a lack of adequate-sized experimental arenas (**Appendix 1**). During June-July 2018, trials were conducted 2-3 times weekly in six 50-gal

($0.19m^3$) aquaria using megalops. A randomized block design was employed wherein each experimental day represented a block with prey densities of 5.3, 10.6, 26.4, 52.8, 264.2, and 528.3 megalops/m3 randomly assigned to a single 50-gal aquarium. Three days of trials were conducted with 90-100 mm FL Chinook during June 9-16, 2018 to emulate the size of juveniles utilizing shoreline habitats prior to shifting into epi-pelagic habitats. Twelve sets of feeding trials were conducted for juvenile Chinook >120 mm FL from June 15 until July 14, 2018 when megalops were no longer available. Prior to experimentation, juvenile Chinook were acclimated to larval crab as food for 24-48 h. The fish were then food-deprived for 24 h, transferred to open-ended vertical acrylic cylinders inside the experimental arenas, and allowed to acclimate for 60 min prior to a trial. Prey were added and allowed to disperse through the arena before juvenile Chinook were released by lifting the open-ended cylinders out of the tank. The consumers were observed, recording the time of initial feeding, after which the trial would run for another 20 min. If no feeding attempts were attempted, the trial was terminated after 30 min. Each trial was terminated by dip-netting the consumers out of the arena, euthanized immediately in an overdose of buffered MS-222 and then dissected. The number of prey found in the mouth, esophagus and stomach was recorded for each actively-feeding consumer and averaged across all trials within each prey density level. All fish handling activities were conducted under the auspices of the USGS Western Fisheries Research Center IACUC Protocol #2008-55.

Alternative Type-I and Type II functional response models were fit to the data with best-model selection aided by AICc (Moss and Beauchamp 2007, Haskell et al. 2017).
The resulting functional response curves can potentially be used to evaluate the sensitivity of feeding and growth by juvenile Chinook to variability in the spatial and temporal availability of exploitable larval crab.

Energy Density of Larval Crab - We measured energy density (% dry mass and J/g wet mass from bomb calorimetry) of crab megalops, focused on *Cancer productus* which was the major contributor to the diet of juvenile salmon. Larval crab were collected with aquarium dip nets along docks in the Union Wharf Marine in Port Townsend and with vertical tows using 300-

micron mesh zooplankton nets from the 0-100 m depth layer during May through July and were frozen until processing. Thawed samples were sorted by size/stage (Z4, Z5 and megalops) by further filtering through larger mesh and hand-picking. Megalopae were graded into 1-mm size bins. Approximately 2 g of fresh or thawed sample material was used for each sampling unit (dry pellet). The size/stage-stratified samples were weighed (blotted wet weight), measured, dried (60^oC for at least 48 h until constant dry weight achieved), reweighed and recorded. Dried samples were ground to a fine powder with a mortar and pestle, homogenized, and pressed into 0.1-g (range 0.05–0.15 g) pellets. The pellets were combusted in a Parr semi-micro bomb calorimeter to measure the energy density (J/g dry weight). The dry weight energy densities were converted back into wet weight energy density units (J/g wet weight) for use in bioenergetics modeling and to provide a measure of energetic profitability of live prey to consumers.

Energy Density of juvenile salmon through the growing season

We measured the energy density of juvenile Chinook salmon as functions of body mass, month, and habitat type (nearshore versus offshore), and region for hatchery and wild fish according to the availability of sufficient frozen archival samples from field collections in estuarine delta, nearshore, and offshore habitats during 2014-2015, supplemented by frozen samples collected in midwater trawls during the R/V *Ricker* cruises in 2016. Other preservation methods altered the energy content of samples and could be used for calorimetry. Sample processing, calorimetry, and analyses followed the methods outlined above for larval crab. Up to 5 fish samples (pellets) were measured per month x region x habitat combination.

The paired % dry Wt and J/g wet weight measures from an initial range of life stages and size categories were regressed to create a calibration curve. Wet weight energy density is commonly highly correlated with % dry Wt (e.g., Ciancio et al 2007). Therefore, a calibration curve with high precision and accuracy could potentially be used to estimate energy density as J/g wet weight from % dry Wt without doing calorimetry.

Results

Spatial-Temporal Variability in Availability of Edible Crab Larvae

The majority of larval crab contributing to the diet were Cancer and Pinnotheridae zoea, and megalopae of Cancrid crab species, predominantly *Cancer productus*, *C. gracilius*; *C. magister* were not observed in the stomach samples, presumably because the megalops of Dungeness crabs were too large to be consumed efficiently by the size of subyearling Chinook that prevailed during the critical growth period. The minimum sizes of both zoea and megalopae in the diets was 1.13 mm carapace length (CL). The mean sizes of megalopae in the diets of Chinook salmon varied among sampling periods and regions, but these differences were not significant (**Figure 1**). The mean (± SD) carapace lengths of megalops in the diet generally ranged within 1.25-3.40 mm. During the critical growth period in June-July, the mean carapace lengths of megalops tended to be larger in Bongo nets than diets (but not statistically so) in the Skagit and Nisqually regions, but smaller in the Bongo nets than diets in Snohomish region (**Figure 1a,b**). The narrow range of sizes available for regressing paired CL and weight (CL: 1.5-2.6 mm; W: 0.008-0.018 g) generated a relatively weak correlation ($r^2 = 0.44$, N = 60):

$$W = 0.0066 \bullet CL^{0.852};$$

therefore, we used the mean CL = 2.1 mm and W = 0.013 g in subsequent computations for biomass and feeding in the sections below.

Temporal-spatial numerical and biomass density of larval crab

After filtering for just the species and size ranges of larval crab found in the diets, prey densities were highly variable through time and among watersheds. In 2014, useable data were available from the Snohomish and Nisqually regions (**Figure 2**). Densities of Z5 Zoea were generally similar or higher than densities of megalops in both basins. The densities of megalops peaked during early June in Nisqually at 12/m^3 (0.152 g/m^3) and late June in Snohomish at 7/m^3 (0.088 g/m^3). During 2015, data for edible species and sizes of larval crab were available for all regions (**Figure 3a-f**). Prey densities varied markedly through time with no apparent coherence among marine regions. Megalops densities ranged from <1/m^3 up to 9/m^3 (<0.013-0.114 g/m^3). The Nooksack region exhibited the earliest, highest, and most consistent densities of megalops, followed by the San Juan Islands. In the two regions with comparable data for both years, the timing and densities of peak availability of megalops in the Snohomish and Nisqually regions were dramatically lower (1.6-3.4/m^3) (0.020-0.043 g/m^3) in 2015 than 2014.

Functional Response of Juvenile Chinook Feeding on Larval Crab

For the smaller 90-100 mm FL Chinook salmon that emulated shoreline-feeding life stages, no relationship between prey density and feeding rate was apparent. For trials where at least one prey was consumed, the average feeding rates of the smaller Chinook salmon (0.23 megalops/min, 2SE = 0.09) appeared slightly lower, but did not differ significantly from consumers > 120 mm FL (0.30 megalops/min; 2SE = 0.12). The smaller juveniles also exhibited an apparent higher rate of unsuccessful strikes at megalops (0.45 strikes/min, 2SE = 0.26, N = 18 trials) than the consumer > 120 mm FL (0.28 strikes/min, 2SE = 0.10, N = 72 trials), but these rates were not significantly different.

For juvenile Chinook salmon > 120 mm FL, feeding rates increased with increasing density of megalops over the range of ambient densities observed in Puget Sound (0-11 megalops/m^3), but then remained relatively constant at higher prey densities (**Figure 4**). This asymptotic feeding pattern conformed to a Type II functional response. The juvenile Chinook showed no significant increase in feeding rate in response to an additional 50X increase above observed densities of megalops, suggesting that they do not require high-density prey patches to support high feeding rates **(Figure 5)**. When forced through the origin, a simple linear equation fit the ascending limb of the functional response curve, which corresponded with the range of ambient prey densities measured in the 0-30 m oblique Bongo net samples (r^2 = 0.988):

$$\text{Megalops eaten/min} = 0.009 + 0.025 \bullet \text{Megalops/m}^3$$

The mean carapace length (CL, mm) of crab megalops available for consumers in these feeding trials was 2.1 mm (1.5-2.6 mm, SD = 0.3 mm) and the mean wet body weight (W, g) was 0.013 g (0.008-0.018 g, SD = 0.002 g).

For a 120-mm FL (17.1 g) Chinook feeding offshore would need to eat a daily ration of 1.1 g/d at 12°C (~50% Cmax) to achieve the observed growth rate during the critical growth period in 2015 (Connelly et al. 2018). Under these conditions, a juvenile would be unable to achieve the required ration at prey densities of less than 4 megalops/m^3 (**Figure 6**). Juvenile salmon feed on zooplankton and other invertebrates predominantly during daylight at early marine life stages, and would therefore need to acquire the full daily ration within the approximately 16 h of daylight and twilight available during June-July. In the scenario above, a consumer would require >40 h of feeding at 1 megalops/m^3 and 17 h at 3 megalops/m^3.

Energy Density of Larval Crab

The energy density of Red Rock Crab megalops was 3384 J/g wet weight (2SE = 259 J/g, N = 5 pellets). This energy density was considerably lower than the default value of 4225 J/g wet weight for megalops and 3395 J/g for Z5 zoea used in previous bioenergetics simulations (Connelly et al. 2018).

Energy Density of juvenile salmon through the growing season

The energy densities of subyearling Chinook salmon remained low throughout the first year of marine growth, and except for the early fry migrants, showed minimal variability among months, habitats, and regions (**Figure 7**). For early fry migrants, those sampled during March and April in estuarine delta habitats of the Skagit River exhibited significantly lower energy densities than fry sampled concurrently in nearshore habitats. The energy densities of fry in the estuarine delta increased through the spring, whereas fry in nearshore habitats started with higher energy densities in March and remained relatively constant through May. The energy densities of juveniles from both estuarine and nearshore habitats converged in May and increased through June.

While large numbers of subyearlings shifted into offshore habitats in June, the energy densities of juveniles remained relatively constant and very similar among individuals sampled in estuarine, nearshore, and offshore habitats during June, July and August (**Figure 8a**), even though the offshore fish were larger (**Figure 7b**) and grew at a faster rate (Gamble et al. 2018). The mean energy density during June-August across all habitats was 4480 J/g (2SE = 78 J/g, N = 92). In September, fish in nearshore habitats showed much lower and more variable energy densities; however, this might have been an artifact of low sample size (N = 2), and the lower mean energy density did not differ significantly from adjacent months due to the high variability.

By October, the juvenile Chinook remaining in offshore habitats of Puget Sound exhibited a modest increase in energy density accompanied by a large increase in body mass, whereas fish remaining nearshore were considerably smaller with marginally, but not significantly lower energy density (**Figure 7a-b**). The energy density offshore in October was marginally higher than during the summer, and was significantly higher than in July, but marginally non-significant compared to June and August.

The empirical measures of juvenile energy density were significantly lower than the default weight-dependent values reported in the Wisconsin Bioenergetics Model (**Figure 8a**; Hansen et al. 1997; Deslauriers et al. 2017). For subyearling Chinook salmon, energy density ED (J/g WW) was poorly correlated with wet weight WW (g) ($r^2 = 0.09$, N = 142, P = 0.0002, 0.5-86.9 g):

$$ED = 4306 + 4.256 \bullet WW$$

The default energy densities from the Wisconsin model averaged 37% higher and ranged 24-45% higher than the empirical estimates reported here.

Subyearling energy densities remained relatively close to a theoretical mortality threshold throughout the growing season (**Figure 7a**). The monthly mean energy densities were below this threshold for early migrant fry during March and April in the estuarine delta and for larger juveniles in nearshore during September.

The energy density of juvenile Chinook salmon was strongly and positively correlated with the proportion dry weight (pDW) of the fish ($r^2 = 0.775$, N = 142, range: 36-201 mm FL, 0.5-86.8 g):

$$ED = -1201 + 26359 \bullet pDW$$

An alternative power function version of this relationship, as reported by Trudel et al. (2007), did not measurably improve the fit ($r^2 = 0.781$):

$$ED = 29866 \bullet pDW^{1.2373}$$

DISCUSSION

This work illuminated knowledge gaps regarding how dynamics in availability and energy density of exploitable prey and temporal energy allocation by Chinook salmon affect foraging success, growth, and bioenergetics during a critical growth period in Puget Sound. We determined that Z5 zoea and megalopae of Red Rock Crab and other *Cancer sp.* except Dungeness *C. magister* represented the primary categories of larval crab that support growth during the critical growth period in epi-pelagic habitats of Puget Sound, especially during June-July. These taxa represented the exploitable fraction of the larval crab assemblage based on their availability in the upper water column and body size relative to the gape-limitations of subyearling Chinook salmon during the critical growth period. Dungeness Crab megalopae were larger than the modal size of range megalopae measured from diet contents of subyearling Chinook sampled from late May through early August in offshore habitats.

The exploitable sizes and taxa of larval crab reduced the apparent pool of larval crab available in the epi-pelagic “prey field” for foraging Chinook salmon and rendered ambient prey densities down to less than 11 megalops/m3, a range of densities that corresponded with the ascending limb of feeding rate as a function of prey density in the function experiments. When the functional response curve was applied to ambient densities and size of exploitable prey, and assuming that the diet was composed of entirely larval crab, subyearling Chinook would have required densities of >3 megalops/m^3 in order to achieve the daily rations that corresponded with the growth observed during the critical growth period in 2015.

When attempting to apply experimentally-derived results to the environment, uncertainties related to whether processes and behaviors have been measured at appropriate scales should be considered. Our 50-gal experimental aquaria were a compromise between more desirable larger arenas that would have enabled lower prey density trials and arenas that allowed the consumers to acclimate readily in trials with reasonable replication. On the field sampling side, the oblique bongo net tows from 30 m to the surface during daylight sampled the appropriate depths and times for foraging by subyearling Chinook salmon in epi-pelagic habitats. However, we cannot discern whether the prey densities estimated from the Bongo net tows resulted from randomly distributed prey or large stretches of empty water punctuated episodically by higher density patches of prey. The functional response results suggested that feeding rates would not increase measurably beyond prey densities >11 megalops/m^3, which corresponded to maximum mean megalops density observed in oblique Bongo net samples across the different regions and half-month sampling periods during 20014 and 2015. However, whether the distributions of prey were random or patchy in lower-density samples could affect the realized foraging success of subyearling Chinook salmon. Some dedicated continuous sampling with an optical or acoustic zooplankton counter with image- and size-recognition capability could address this important question of how density and distribution of key prey are scaled in the natural environment.

In the longer term, further development of these functional relationships and associated field monitoring programs will provide tools for application to future zooplankton and juvenile salmon/forage fish monitoring data that could estimate seasonal and regional growth potential (e.g., Brandt et al. 1992) during critical growth periods for juvenile Chinook salmon that could then be used to predict relative marine survival. Additional scenarios could also be simulated to project how future changes in prey availability or thermal conditions might influence growth and survival. This approach would be readily adapted to other juvenile salmon and key forage fish species which would then enable estimates of carrying capacity for the community of epi-pelagic planktivorous fishes during critical periods of growth and survival through the ontogeny of multiple species (e.g., Hansen et al. 2016; Sorel et al. 2016; Haskell et al. 2017). This approach could provide a mechanistic indicator for food availability during critical growth periods and would contribute to a long term monitoring program for both marine survival of salmon and the general forage base for juvenile salmon and forage fishes. This analysis would inform research on environmental and ecological drivers affecting bottom-up and top-down control of juvenile salmon, forage fishes, and their key food resources.

Different life stages of salmon are confronted by different time-sensitive pressures related to growth, survival, migration, or reproduction to which they can respond behaviorally or by shifting their energy allocation among somatic growth, activity, lipid stores, or gonadal investment. For subyearling salmonids, strong size-selective mortality creates a two-pronged challenge: near-term predation mortality by gape-limited predators versus over-winter mortality due to energy depletion or other size-selective process. Investing in faster somatic growth generates larger low-energy content body size that can reduce both acute near-term and more chronic predation risk by quickly outgrowing the most numerous gape-limited predators (Beauchamp et al. 2007). However, this strategy might not sequester sufficient energy stores to withstand prolonged periods of food limitation over the winter. Subyearling Rainbow trout exhibited highly plastic behavioral and energy allocation responses to experimentally-manipulated food availability and predation risk in a series of whole-lake studies, wherein juveniles that experienced good feeding conditions with low predation risk increased both body size and lipid stores throughout the season, whereas subyearlings that were under high threat of predation initially allocated energy into somatic growth to reduce initial acute predation mortality, then back-filled with lipid storage later in the summer in order to survive the winter (Biro et al. 2005). In the high predation risk-low food treatments, subyearling trout were forced to adopt more risky behavior in order to both grow and acquire sufficient lipid stores before winter. In the Puget Sound Chinook situation, we hypothesized that subyearlings would adopt the fast growth, low lipid storage strategy through at least the June-July critical growth period, then increase lipid stores during the latter portions of summer and autumn.

Contrary to our expectations, energy densities remained relatively low at the end of the growing season in October or November rather than increasing significantly before winter. The substantial increase in body mass over the entire growing season, coupled with a very modest increase in energy density by early October resulted in higher overall energy content in the bodies of the juveniles going into winter; however, the implications of this energy status for overwinter survival is yet to be determined. The subyearling Chinook that still remained in Puget Sound by October-November were more likely to be individuals that adopted the resident “blackmouth” life history strategy and might not reflect the energy status of subyearlings that migrated out of Puget Sound earlier. Duffy and Beauchamp (2011) suggested that the emigration from Puget Sound by ocean-bound subyearlings was already underway by late summer, based on length frequency comparisons of samples from late September to earlier months and a much weaker relationship between body mass and overall marine survival (smolt-adult returns, SARs) in September compared to the relationship in July. Trudel et al. (2009) also reported interceptions of subyearling Puget Sound-origin Chinook salmon by September along the west coast of Vancouver Island, based on coded wire tag (CWT) recoveries, and ocean migration distances remained within a similar range of 100s of km from the location of hatchery release through the following year of ocean growth. Comparable energy density measurements for Puget Sound-origin subyearlings captured outside the basin (i.e., in the Strait of Georgia or along the Pacific coast) during later periods during the first summer-fall of growth or during winter would provide valuable insights into the dynamics of seasonal energy allocation and implications for marine survival.

Bioenergetics models are energy-balance equations, and are thus sensitive to errors or real changes in the energy densities of both the consumer and its prey. This study generated considerably lower estimates for the energy density of subyearling Chinook salmon than had been used as default values in commonly-used bioenergetics models (Hansen et al. 1997; Deslauriers et al. 2017). Thus, bioenergetics simulations would overestimate consumption rates by approximately 37-40% higher values if fitting the consumption required to satisfy observed growth using the default energy density values for Chinook Salmon. The calorimetry estimates for energy density of crab megalops yielded values that were substantially lower than previously used (3384 J/g versus 4225 J/g). By reducing the energy density of crab larvae will cause the bioenergetics model estimates of consumption to increase by approximately 30% during periods when crab larvae are a major contributor to the diet. Fortunately, these models allow user-defined energy density scenarios for both the consumer and prey, so these findings can be readily incorporated into future modeling simulations.

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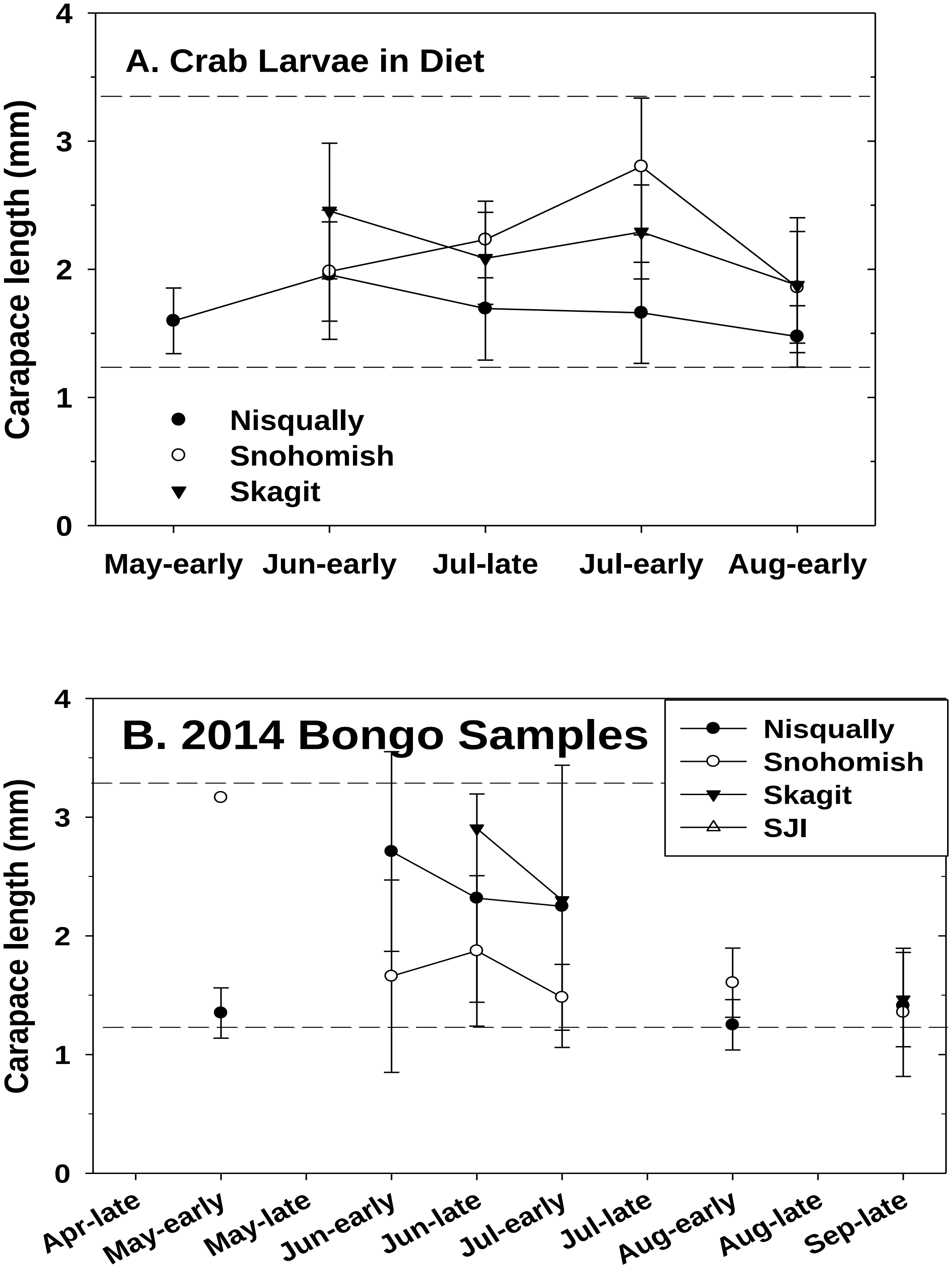


Figure 1. **A**) Mean carapace length (±1 SD) of crab megalops, primarily *Cancer productus*, measured in diets of juvenile Chinook salmon; and **B)** sampled concurrently in oblique Bongo net tows over 0-30 m depths in different sampling periods and regions during 2014. Dashed reference lines bound the size range of larval crab in diets.

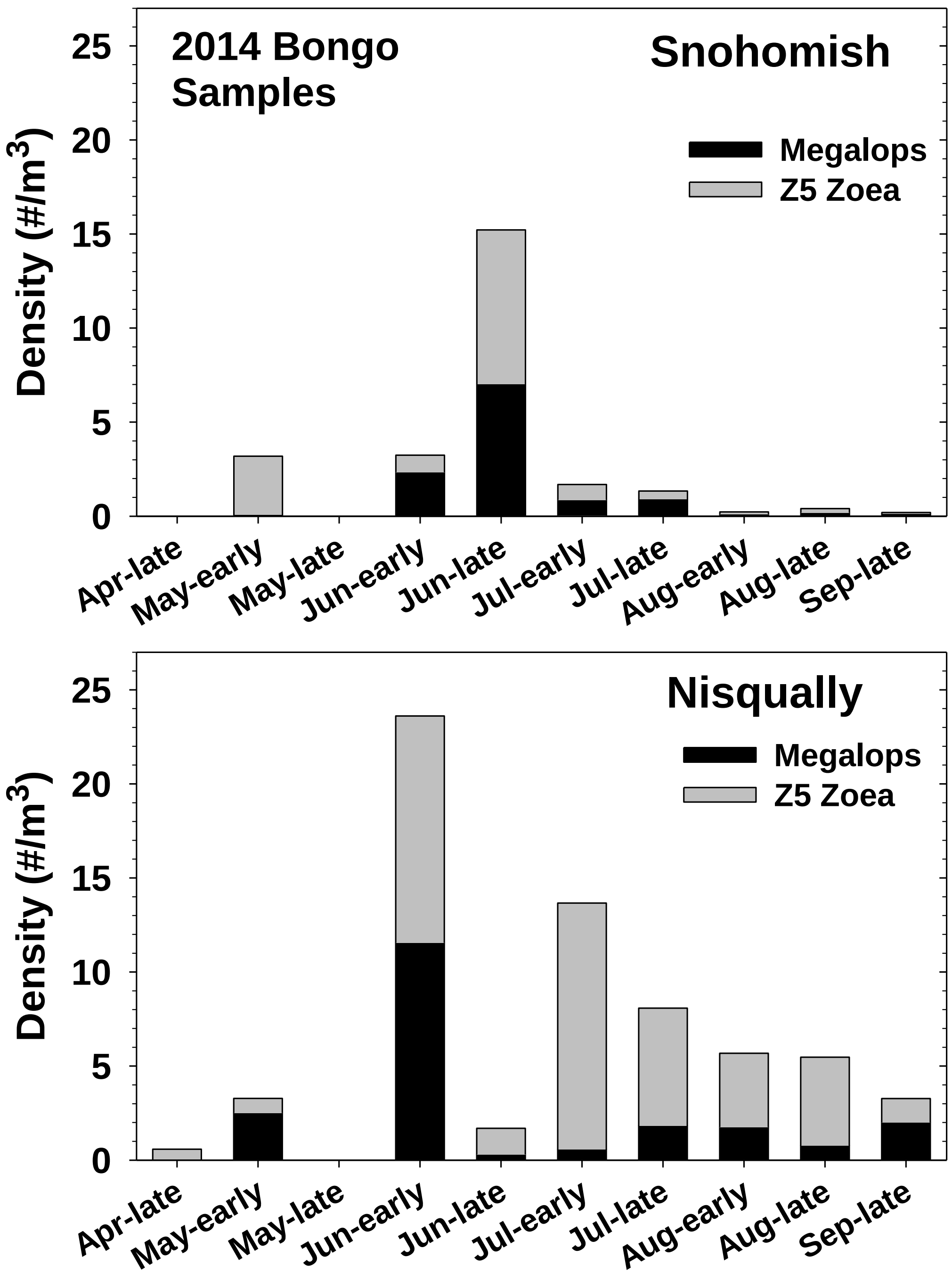


Figure 2. Densities of the sizes and species of crab zoea (Z5) and megalops measured in twice-monthly oblique bongo net tows from 30 m deep to the surface in the offshore marine habitats of the Snohomish and Nisqually regions.

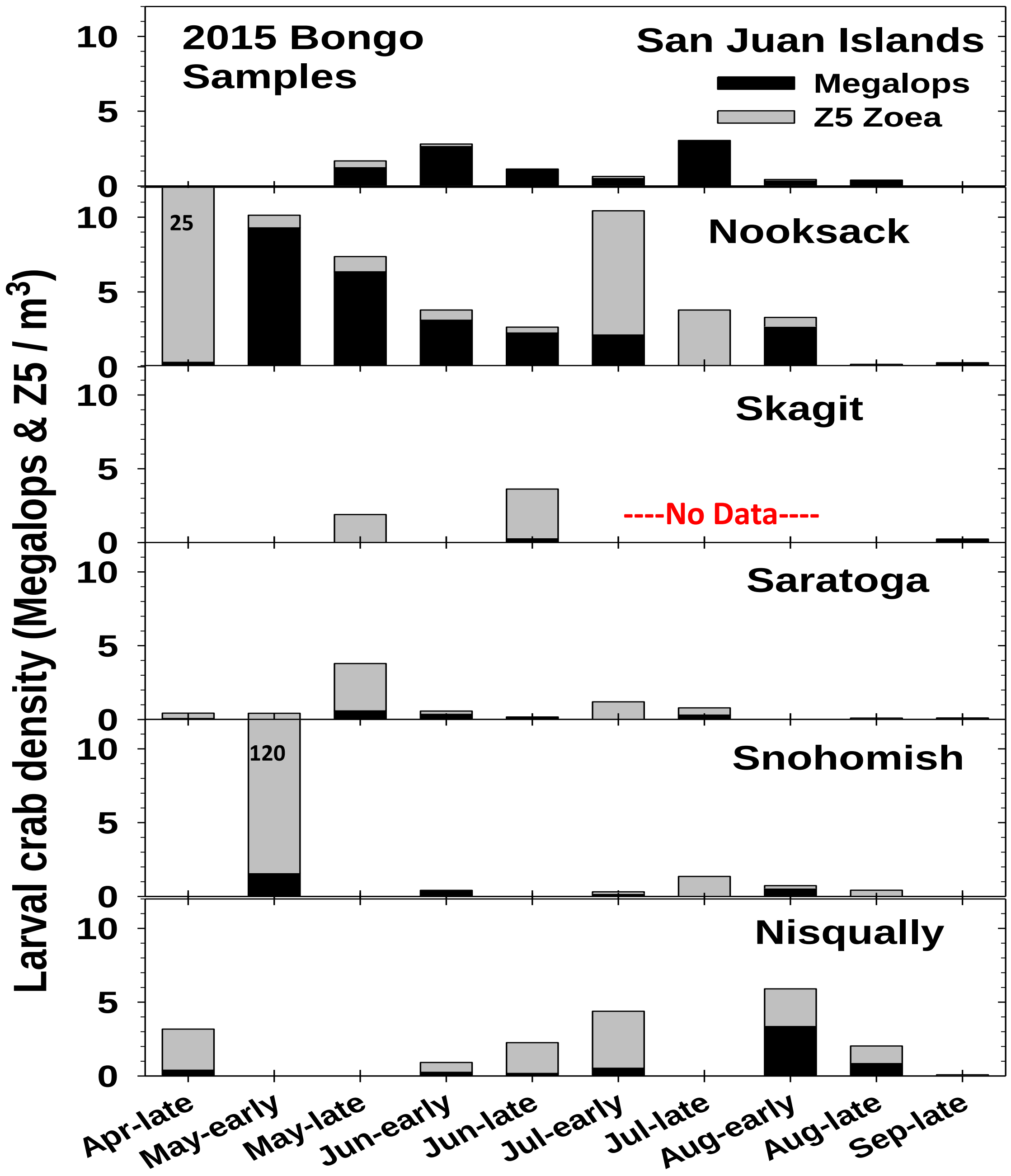


Figure 3. Densities of the sizes and species of crab zoea (Z5) and megalops measured in twice-monthly oblique bongo net tows from 30 m deep to the surface in the offshore marine regions of Puget Sound during spring-summer 2015.

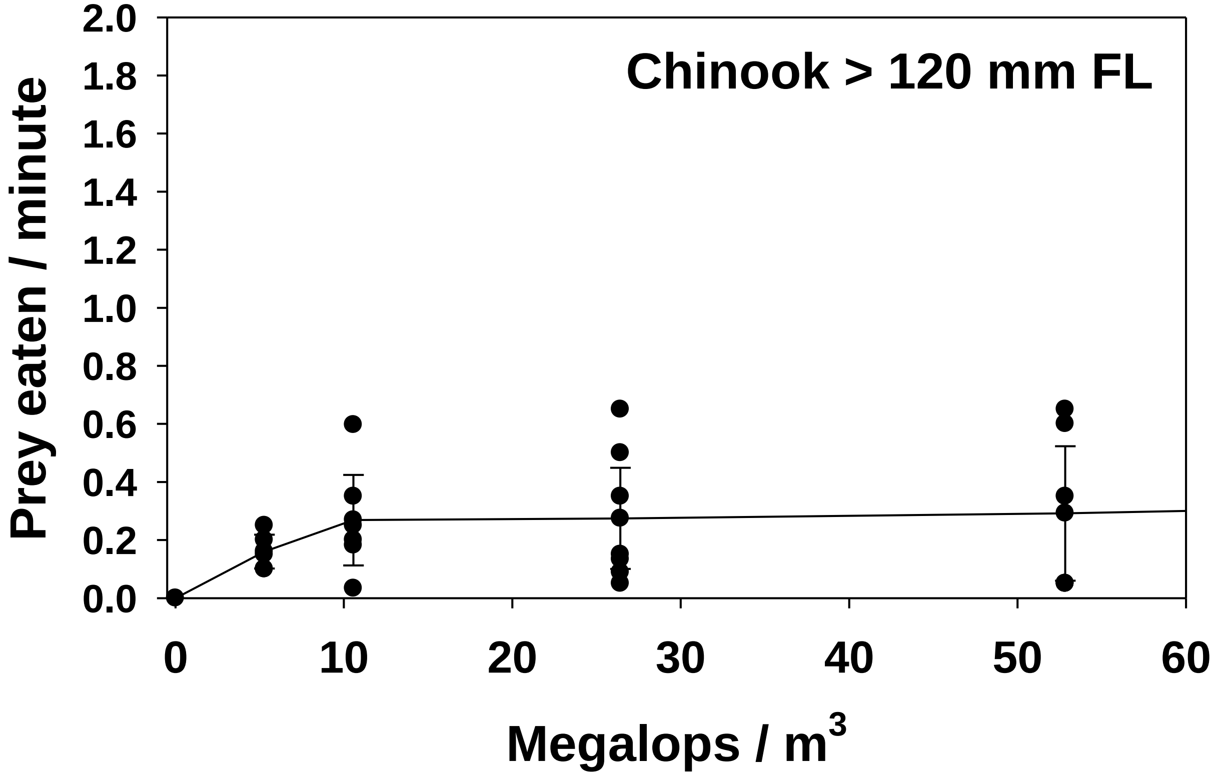


Figure 4. Functional response of juvenile Chinook salmon > 120 mm FL to varying densities of crab megalops. The same data are plotted in all three panels, except that the middle and lower panels include much higher densities than the averages observed from oblique bongo net tows through 0-30 m depths during the critical growth period. Lines connect the mean feeding rates and error bars represent 2SE.

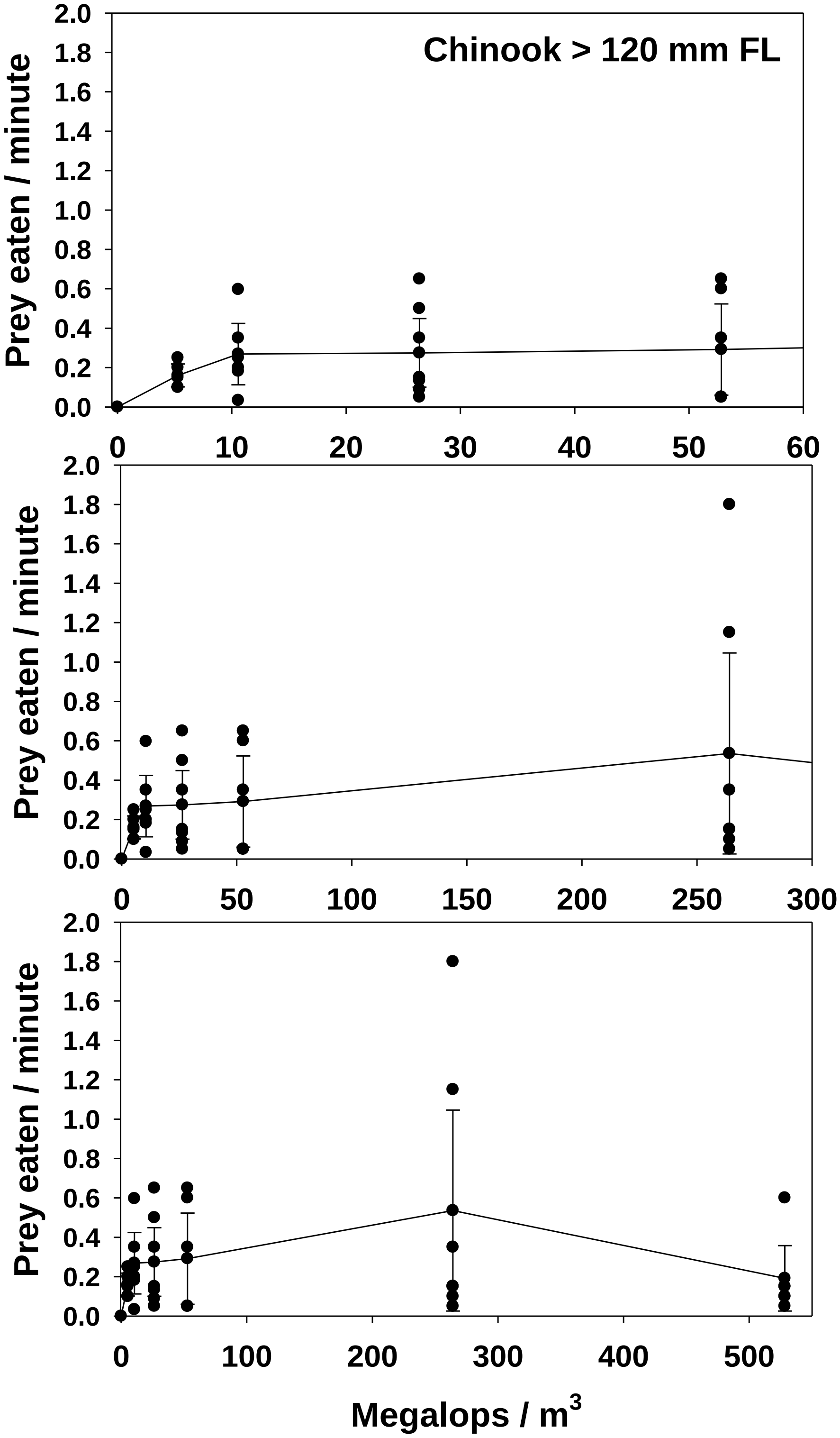


Figure 5. Functional response of juvenile Chinook salmon > 120 mm FL to varying densities of crab megalops. The same data are plotted in all three panels, except that the middle and lower panels include much higher densities than the averages observed from oblique bongo net tows through 0-30 m depths during the critical growth period. Lines connect the mean feeding rates and error bars represent 2SE.

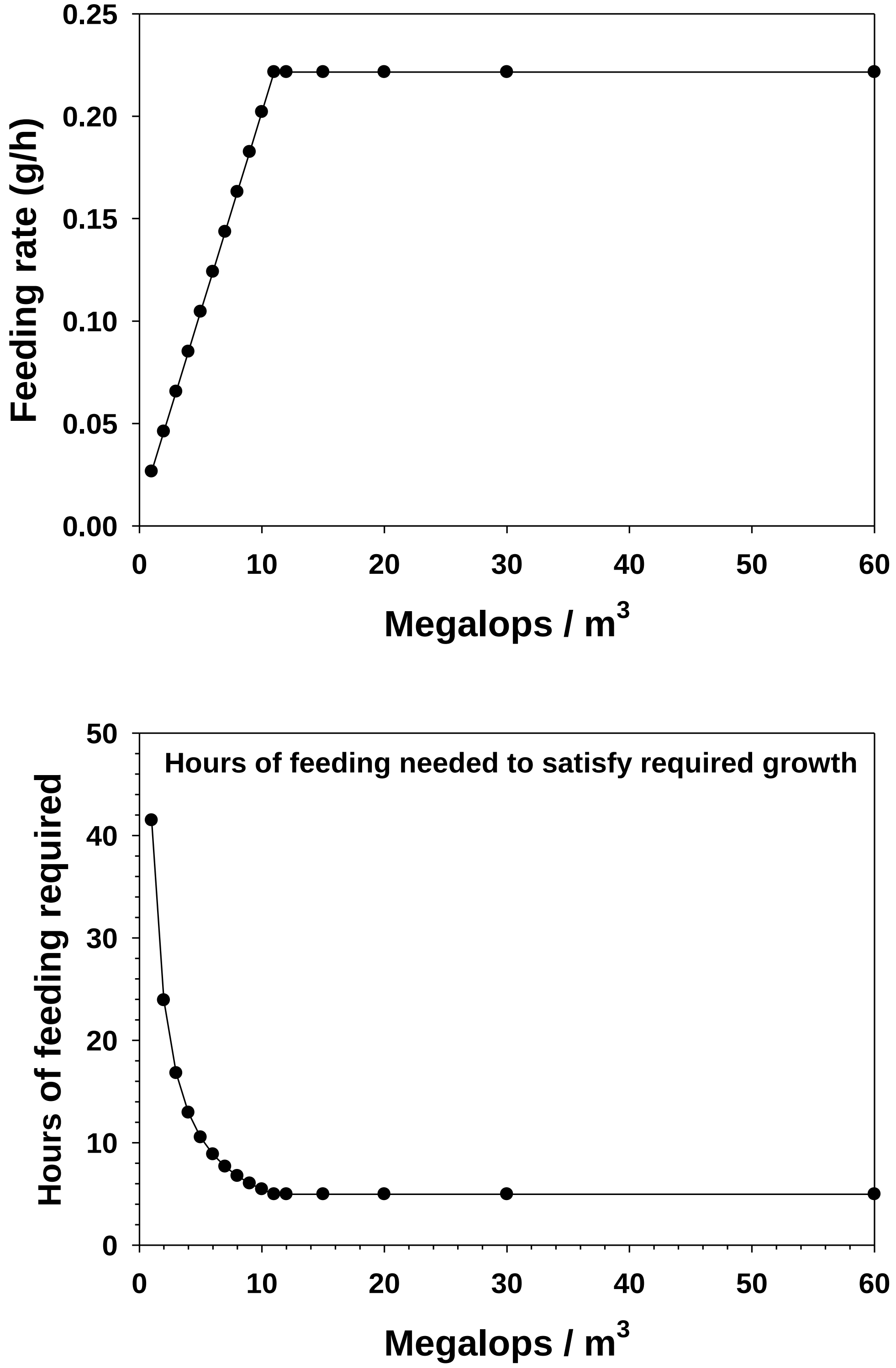


Figure 6. The estimated feeding rate (in g/h) of a 120-mm FL Chinook (17.1 g) as a function prey density (top panel); and (lower panel) the hours of foraging required to reach the approximate ration required to achieve observed growth (50% Cmax) at a thermal experience of 12ºC.

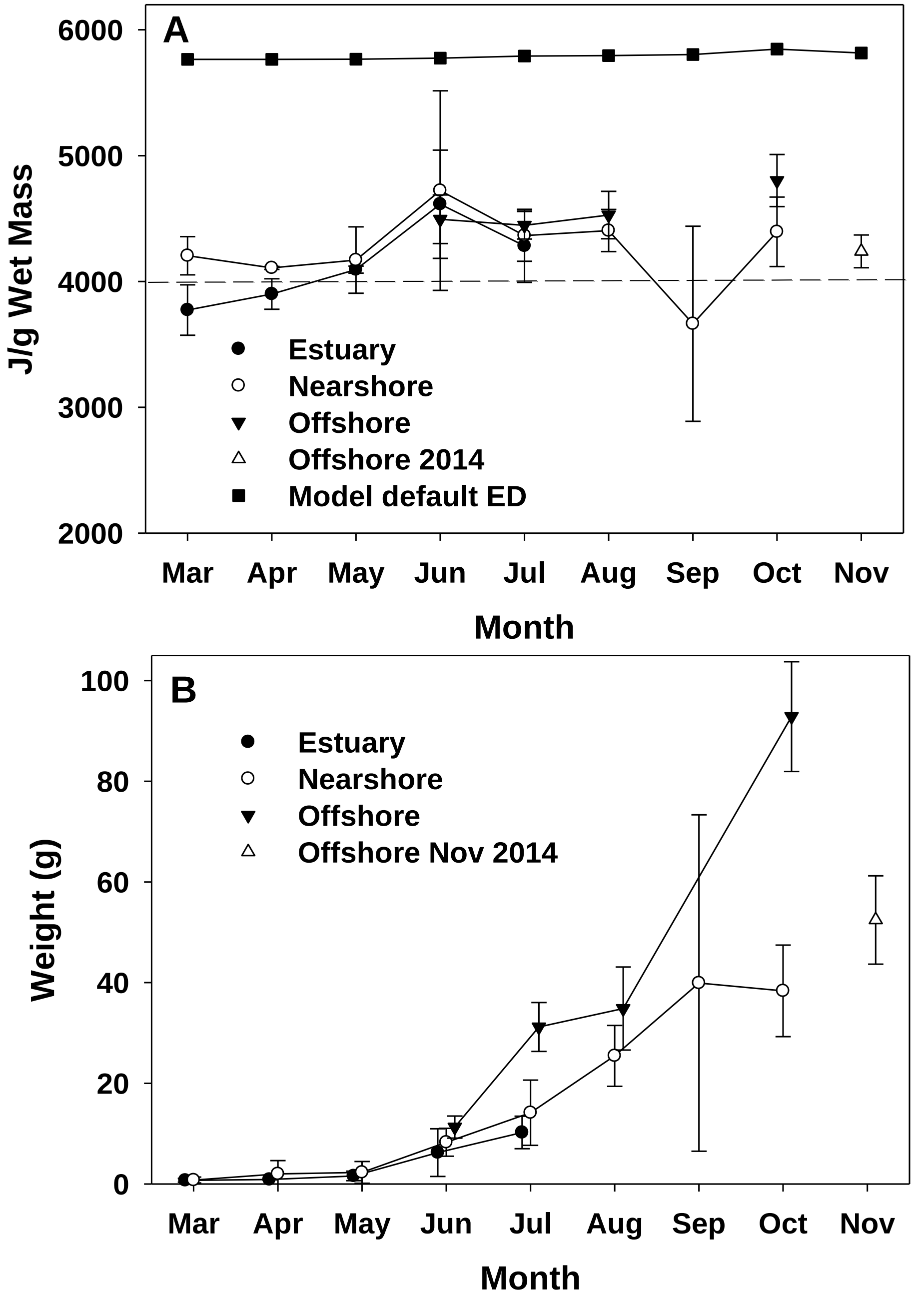


Figure 7. **A-**Monthly mean (± 2 SE) energy densities of subyearling Chinook Salmon sampled from estuarine delta, nearshore, and offshore habitats within Puget Sound during 2015 and measured by bomb calorimetry (upper panel). The samples from November were collected in 2014 (by midwater trawling with the *RV Ricker*), when fish were significantly smaller than in 2015. For comparison, the default energy density values used by the Wisconsin bioenergetics model (uppermost curve) significantly overestimated energy densities for the corresponding mean monthly body masses of juvenile Chinook. The dashed line represents the theoretical energy density threshold associated with mortality. **B-**Monthly mean body weights (± 2 SE) of subyearling Chinook samples used for calorimetry, by habitat in 2015 and November 2014 offshore. Although similar to the full spatial-temporal complement of subyearlings sampled in 2014-2015, these fish might not accurately represent the mean size or variability within and among habitats and months due to the low sample sizes used for calorimetry.

APPENDIX 1

2017 Functional Response Trials

During 2014, the range of low prey densities were constrained by the size and type of experimental arenas that did not inhibit feeding behavior by the juvenile Chinook salmon. We were originally planned for feeding trials to be conducted in neutral gray 1-m^3 circular fiberglass tanks. However, the juvenile Chinook never acclimated to these arenas consistently, despite testing numerous combinations of acclimation periods, pre-conditioned feeding with larval crab, and single or multiple consumers per arena. The fish fed readily in glass aquaria. Consequently, in 2017, the feeding trials were conducted in nine 10-gal (0.038 m^3) aquaria. Unfortunately, the 10-gal aquaria were the largest arenas we could obtain in time to conduct replicated experiments during the time window when appropriate sizes and taxa of larval crab were available. This limited the minimum prey density levels, because a single prey per arena represented 26 megalops/m3, a density that was more than double the maximum densities estimated from oblique Bongo net samples in 0-30 m epi-pelagic depths across the major basins in Puget Sound during June-July 2014 and 2015.

In addition, Chinook never reached the size of juveniles observed in offshore habitats (**Figure A1**), thus calling into question the relevance of any results from these trials.

A generalized fork length-weight regression was generated by Beauchamp and Duffy 2011 (r^2 = 0.99, N = 57; FL range: 42-200 mm, W range: 0.65-79.00 g):

$$W(g) = 0.0000049 \cdot FL(mm)^{3.147}$$

Was used to generate a generalized estimate of body mass for a 120-mm FL subyearling Chinook feeding offshore during the critical period when applying the functional response to ambient prey densities reported in Puget Sound during spring-summer 2014 and 2015.

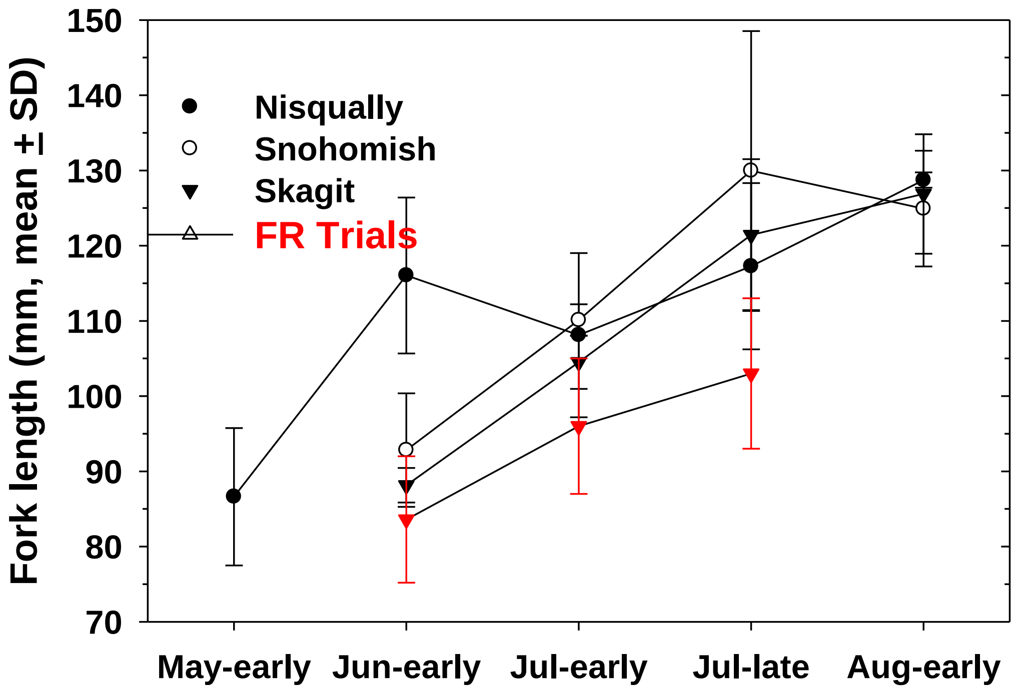
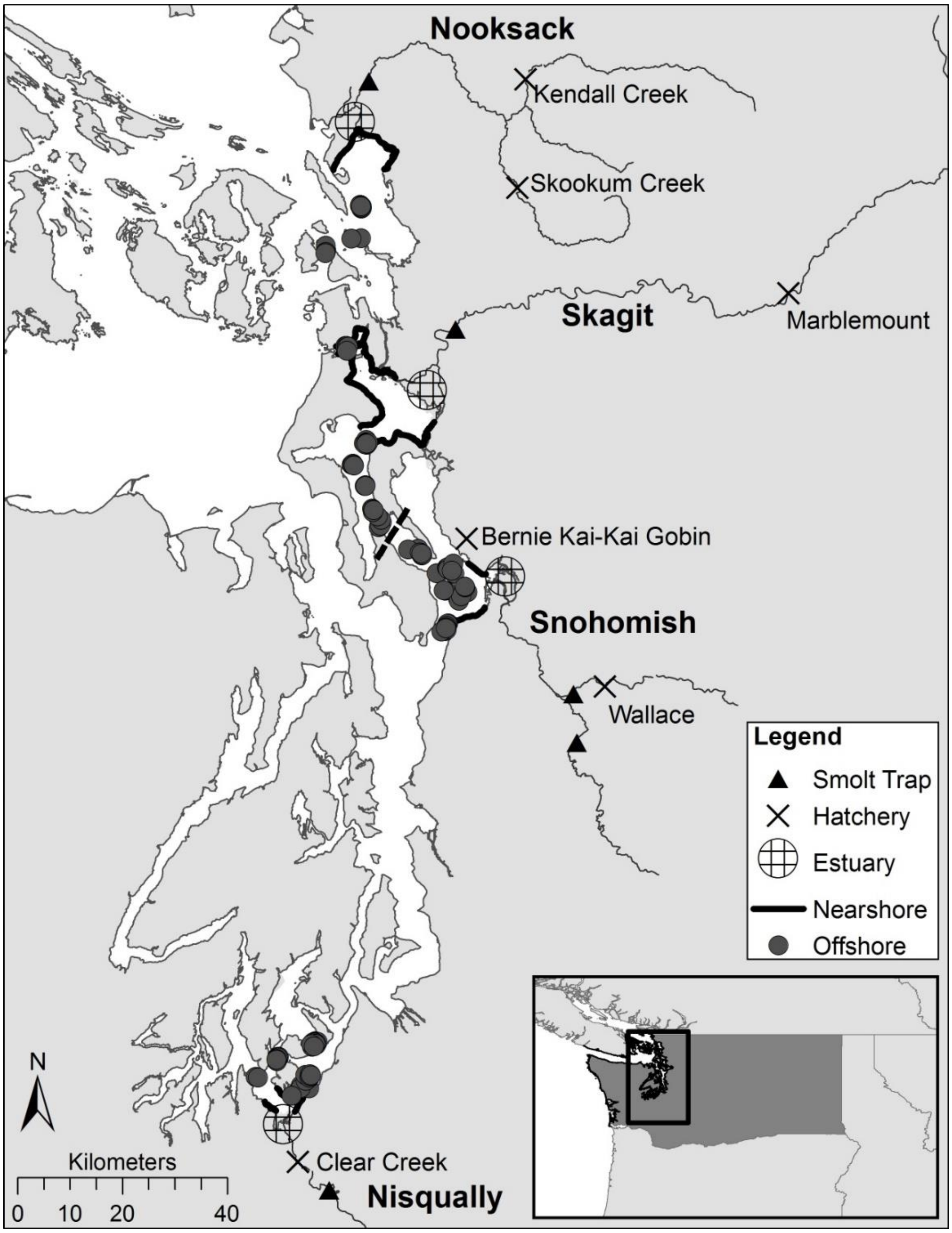


Figure A1. The mean fork length of juvenile Chinook available for feeding rate trials in 2017 (red solid upside down triangles) compared to the sizes measured for juveniles sampled in nearshore (May-June early) and offshore (July-Aug) habitats in different regions of Puget Sound during 2014-2015.



Map showing approximate locations of juvenile Chinook salmon sampling efforts in each habitat and focal watershed in Puget Sound, WA during 2014 and 2015. The black dashed line differentiates between what was considered the offshore region of the Skagit and Snohomish watersheds.