# GROWTH AND TROPHIC ECOLOGY OF JUVENILE DUNGENESS CRABS IN THE SOUTH SLOUGH ESTUARY

by

MICHAEL THOMAS

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Student: Michael Thomas

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This thesis has been accepted and approved in partial fulfillment of the requirements for the Master of Science degree in the Department of Biology by:

| | |
|---|---|
| Alan Shanks | Chairperson |
| Aaron Galloway | Member |
| Bree Yednock | Member |

and

| | |
|---|---|
| Janet Woodruff-Borden | Vice Provost and Dean of the Graduate School |

Original approval signatures are on file with the University of Oregon Graduate School.

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# THESIS ABSTRACT

Michael Thomas

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Estuaries serve as important nursery habitat for young Dungeness crabs throughout their range in the northeastern Pacific. To better understand the function of small estuaries as nursery habitat for juvenile and sub-adult Dungeness crabs, we conducted a multi-year survey of crabs within the South Slough estuary on the southern Oregon coast to explore spatiotemporal patterns in how they use the estuary. Additionally, we used laboratory feeding assays to investigate the use of fatty acids (FA) as biomarkers in juvenile Dungeness crabs and compared the FA composition of juvenile crabs collected in the South Slough estuary to those fed controlled diets in the laboratory. We found that the South Slough estuary functions as important nursery habitat for juvenile crabs and observed that a limitation of high-quality food may contribute to slow juvenile growth during years of massive settlement.

This thesis includes previously unpublished co-authored material.

# CURRICULUM VITAE

NAME OF AUTHOR: Michael Thomas

GRADUATE AND UNDERGRADUATE SCHOOLS ATTENDED:

University of Oregon, Eugene
Northern Arizona University, Flagstaff

DEGREES AWARDED:

Master of Science, Biology, University of Oregon
Bachelor of Science, Education , Northern Arizona University

AREAS OF SPECIAL INTEREST:

Marine Biology

PROFESSIONAL EXPERIENCE:

Teaching Assistant, Department of Biology, University of Oregon, 2017-2019

Research Assistant, Department of Biology, University of Oregon, 2016-2019

GRANTS, AWARDS, AND HONORS:

Oregon Dungeness Crab Commission, 2017-2019

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For my father, who instilled in me a life-long passion for learning.

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# CHAPTER I

# INTRODUCTION

Chapters II and III of this text include previously un-published, co-authored material.

Dungeness crabs (*Metacarcinus magister*, Dana (1852); formerly *Cancer magister*) are a valuable commercial species and ecologically important predator in the northeast Pacific and can be found from Santa Barbara, California to the Pribiloff Islands, Alaska (Rasmuson 2013). Estuaries are important habitat for the early life stages of many species, including young Dungeness crabs (Heck and Thoman 1984, Stevens and Armstrong 1984, Bottom et al. 1988, Beck et al. 2001). Tidal channels, sea grass beds, and salt marshes within estuaries provide complex habitat that provide shelter and foraging grounds (Beck et al. 2001). There is ample evidence for the nursery function of estuaries for young Dungeness crabs (Carrasco et al. 1985, Dumbauld et al. 1987, Gunderson et al. 1990, Fernandez et al. 1993b, Armstrong et al. 1995). The role of estuaries as nurseries for Dungeness crabs is confounded, however, by the lack of estuarine habitat in the northeastern Pacific when compared to estuaries in the western Atlantic (Basta et al. 1990) whose nursery function for other decapod crustaceans, like blue crabs, is well known (Heck and Thoman 1984).

Most estuaries on the west coast of North America are relatively small (< 100 $km^2$) with notable exceptions being Gray's Harbor (WA), Willapa Bay (WA), Puget Sound (WA), Columbia River estuary (WA/OR), and San Francisco Bay (CA) (Emmett et al. 2000). While these larger estuarine systems have been well studied, it is less clear how the relatively small estuarine areas contribute to the life history of Dungeness crabs.

Although smaller estuaries may be limited in their contribution to adult populations in terms of overall numbers, they may provide significant benefits to crabs on a per unit of available habitat basis. For this reason, the ecological importance of Dungeness crabs within these esturies as voracious omnivores (Gotshall 1977, Jensen and Asplen 1998) and even as important food subsidies for higher level consumers (Armstrong et al. 1995) should not be overlooked.

A potentially important part of Dungeness crab ecology is how estuaries contribute to their juvenile diet. The settlement of Dungeness crab megalopae in estuarine systems and the food habits of juvenile crabs may represent an important link between estuarine and nearshore energy flux as crabs reach maturity and migrate from estuaries to the outer coast. Previous studies of juvenile Dungeness crab diets were based on inspection of stomach contents (Gotshall 1977, Stevens et al. 1982), but these methods are biased to prey items with recognizable hard-parts and may miss important soft-bodied sources of nutrition (Pond and Sargent 1998). This is especially true for small crabs, like juvenile Dungeness, who use their claws and mandibles to tear and masticate their food prior to ingestion. For these reasons, molecular "biomarkers" that can provide information about an organism's diet are an attractive option. Fatty acids (FA) are one such biomarker and have been used in trophic investigations for decades (Dalsgaard et al. 2003). Biomarkers are not subject to the same biases as gut content analyses, although they do have their own limitations (Dalsgaard et al. 2003, Kelly and Scheibling 2012). The ability of some invertebrates to significantly modify or preferentially assimilate dietary FA creates a challenge for using FA as trophic markers (Bell and Tocher 2009)

and demonstrates the need to use multiple markers paired with controlled feeding assays when making trophic inferences about consumers (Galloway et al. 2015).

Here we present the results of monthly sampling during four settlement seasons (2015-2018) at six stations within a small estuary on the southern Oregon coast (South Slough) to study spatiotemporal patterns in how juvenile and sub-adult Dungeness crabs use the estuary. Additionally, we investigated the diet of wild 0+ juvenile Dungeness crabs in South Slough using multivariate FA analyses and compare the results with two controlled feeding assays of early instar juveniles fed a variety of mono-specific diets.

# CHAPTER II

# GROWTH AND ABUNDANCE OF JUVENILE AND SUB-ADULT DUNGENESS CRABS IN THE SOUTH SLOUGH ESTUARY

This chapter includes previously unpublished, co-authored material. The experimental design and field sampling were performed by Bree Yednock, PhD and the staff at the South Slough National Estuarine Research Reserve (SSNERR). Alan Shanks, PhD and Aaron Galloway, PhD provided advice regarding data analysis and the preparation of the manuscript. I was responsible for the data analysis and writing of the manuscript.

**Abstract**

Estuaries serve as important nursery habitat for young Dungeness crabs throughout their range in the northeastern Pacific and many studies have demonstrated this within large estuarine systems. Little is known, however, about how the abundant small, coastal estuaries contribute to the life history of this commercially and ecologically important species. To better understand the function of small estuaries as nursery habitat for juvenile and sub-adult Dungeness crabs, we sampled juvenile crabs using monthly beach seines between July 2015 and November 2018 at six sites within a small estuary (South Slough) on the southern Oregon coast and used a light trap at the mouth of the estuary to collect larval crabs as an index of settlement. Settlement, as measured by number of megalopae caught daily in the light trap throughout the settlement season, varied annually by almost three orders of magnitude (3,106 in 2016 and 2.8 million in 2018). Juvenile young-of-the-year crabs (0+) in the estuary grew rapidly in their first summer and attained an average size of 62 mm carapace width (CW) six months after

settlement. Crab catches declined by fall of their second year when they averaged 127 mm CW. Between 2015 and 2017, average summer/fall catch per unit effort ranged from 119 – 314 / ha, but CPUE was significantly higher in 2018, when massive settlement of larvae increased density almost ten-fold (2,071/ha). Growth of 0+ crabs was similar in all years except in 2018 when densities were high. By November of 2015-2017, the average CW of 0+ crabs ranged from 62.3 mm to 67.6 mm. In 2018, average CW of 0+ crabs was only 51.5 mm CW. Our study indicates the South Slough serves as important nursey habitat for young Dungeness crabs and in years when larval settlement in high, density dependent effects may reduce 0+ growth.

**Introduction**

Estuaries are critical habitat for the early life stages of many species, and the role of estuarine habitat as a nursery for both vertebrate and invertebrate species has been well documented (Heck and Thoman, 1984; Stevens and Armstrong, 1984; Bottom et al., 1988; Beck et al., 2001). Tidal channels, sea grass beds, and salt marshes within estuaries provide complex habitat that serve not only as shelter, but also foraging grounds (Beck et al., 2001). The designation of juvenile habitat as "nursery" grounds has not been consistently applied nor always rigourously tested. In some cases the term has been assigned to habitat where juveniles are found in high densities or are afforded some apparent advantages without any comparison to other juvenile habitat. Beck et al. (2001) noticed this and provided a clear, testable definition of nursery habitat as one that produces more indivuals per unit area that ultimately recruit into the adult population, when compared to other habitats where juveniles occur. Contributing more individuals per unit area can result from any combination of four factors: 1. density, 2. growth, 3.

survival, and 4. movement to adult habitat (Beck et al., 2001). High densities of juvenile Dungeness crabs, *Metacarcinus magister* (Dana, 1852; formerly *Cancer magister*), have long been observed in estuaries (Emmett and Durkin, 1985; Orcutt, 1977) and this habitat was presumed to serve a nursery role. The nursery function of estuarine habitat for Dungeness crabs has now been tested and ample evidence indicates the young-of-the-year (0+) grow faster (Carrasco et al., 1985; Dumbauld et al., 1987; Gunderson et al., 1990), are able to use structured habitat to avoid predation and increase survival (Fernandez et al., 1993b; Armstrong et al., 1995), and have access to more available food when compared to juveniles that settle in the coastal ocean (Gunderson et al., 1990).

The role of estuaries as nurseries for Dungeness crabs is confounded by the apparent lack of estuarine habitat in the northeastern Pacific when compared to estuaries in the western Atlantic (Basta et al., 1990) whose nursery function for other decapod crustaceans, like blue crabs, is well known (Heck and Thoman, 1984). Dungness crabs are found in the eastern Pacific from Santa Barbara, California to the Pribiloff Islands, Alaska (Rasmuson, 2013). There are many estuaries within this range, but most are relatively small (< 100 $km^2$) with a few notable exceptions being Gray's Harbor (WA), Willapa Bay (WA), Puget Sound (WA), Columbia River estuary (WA/OR), and San Francisco Bay (CA) (Emmett et al., 2000). While estuaries clearly offer distinct advantages to juvenile and sub-adult Dungeness crabs, it is less clear how the relatively small estuarine areas found in their range contribute to adult populations. Armstrong et al. (2003) estimate the contribution of estuarine production of Dungeness crabs to adult populations can be 25-30% in large systems (Willapa Bay, Gray's Harbor & Columbia River) while smaller systems provide a much smaller 5-7% (Coos Bay, Yaquina Bay).

Although the contribution of smaller estuarine systems may not add large numbers to adult populations, the ecological importance of Dungeness crabs within these esturies as voracious omnivores (Gotshall, 1977; Jensen and Asplen, 1998) and as important food subsidies for higher level consumers (Armstrong et al., 1995) should not be overlooked. Dungeness crabs can settle in very high densities and may represent a significant annual disturbance in estuarine systems (Galloway et al., 2017).

Dungeness crab settlement is strongly influenced by abiotic forces and annual abundances of returning larvae and settled juveniles can vary by serveral orders of magnitude (Hobbs et al., 1992; McConnaughey and Armstrong, 1992; Shanks, 2013). These variations apply not only to the annual abundance of new settlers, but also to the timing of their delivery as larvae within each settlement season (Miller and Shanks, 2004). The implications of annual and within-season variability on the success of young-of-the-year crabs is not well understood. Commercial landings of Dungeness crab have been used as a tool to assess population dynamics and wide fluctuations in the commercial catch have been correlated with a variety of hydrodynamic and atmospheric variables (Botsford and Wickham, 1975; McConnaughey and Armstrong, 1992; Shanks, 2006). Early work focused on how abiotic factors affecting the larvae impacted year class strength (Jamieson and Armstrong, 1991). More recently, there is evidence that post-settlement density-dependent effects may dominate when the number of returning larvae is large (Eggleston and Armstrong, 1995; Shanks, 2013). Shanks (2013) used a 12 year time series of the number of megalopae arriving in the Coos Bay estuary (OR) to show that when abiotic conditions result in high settlement, the commercial catch may be impacted by density-dependent effects years later. Additionally, in field experiements

conducted in Willapa Bay, Fernandez (1993a) observed that early season settlers reduced the abundance of subsequent settlers in the same recruitment season and proposed cannibalism as a possible explanation.

A great deal of work has described the growth, abundance, and habitat utilization of juvenile and sub-adult Dungeness crabs in the larger eastern Pacific estuaries (San Francisco Bay, Willapa Bay, & Grays Harbor), but little is known about how these factors compare in smaller estuaries. Here we present the results of monthly sampling during four settlement seasons (2015-2018) at six stations within a small estuary on the southern Oregon coast (South Slough). We are aware of only one similar study in this estuary (Rooper et al., 2002), however, this work was part of a broad scale analysis of Pacific Northwest estuaries and sampling occurred only in the marine dominated part of the South Slough at a limited temporal scale. We discuss our results in context of observations in other Pacific Northwest estuaries and discuss the impact of a massive crab settlement event observed in 2018 on cohort success within the South Slough.

## Materials and Methods

### *Study Locations*

The South Slough estuary, located on the southern Oregon coast, is a sub-unit of the larger Coos Bay estuary (the largest in Oregon), and has a wet surface area of 783 ha (Rumrill, 2006). The narrow mouth of the South Slough is adjacent to Charleston, OR and is just 3 km from the mouth of the Coos Bay estuary (**Figure 1**). The South Slough, a north-south trending channel, is marine-dominated at its north end and riverine to the south. The southern half of the Slough is designated as the South Slough National

Estuarine Research Reserve (SSNERR), the first National Estuarine Research Reserve (NERR) in the United States.

Sampling took place at six stations within the South Slough that had been previously used to monitor spatio-temporal patterns in fish assemblages (Bottom et al., 1988). The crabs sampled for this study were collected as part of larger project comparing fish assemblages to the surveys of Bottom et al. (1988). Stations were located from near the mouth of the estuary to well inside each of the two major arms of the slough (**Figure** 1). Each station consisted of mostly unvegetated benthos to minimize seine net fouling and habitat destruction, although, ephemeral algae (*Ulva spp.*) and eel grass (*Zostera marina*) were occasionally present. Station A was a mostly sandy bottom habitat, while the remaining stations had a fine, silty mud bottom. For comparisons between sites we grouped stations into four categories that describe their spatial position within the slough: 1. Close to the mouth of the estuary (Stations A & B), 2. Middle estuary (Stations C & D), 3. Upper estuary, Winchester arm (Station E), and 4. Upper estuary, Sengstacken arm (Stations F) (**Figure** 1).

*Sampling Procedure*

Monthly beach seines were collected from each station between July 2015 and November 2018. The seine net was deployed by boat and replicate seines were collected, when possible, at both high and low tide at all sites except Stations E & F (upper estuarine sites), which were only sampled at high tide because it was not possible to access these two stations by boat at low tide. The high tide seines sampled the mudflats and fringing marsh areas, while low tide collections sampled within the channels or lower mudflats and fringing eelgrass beds at each site. The seine net was 37 m x 2.4 m (L x H)

with an 8 mm mesh and a 2.4 m x 2.4 m central capture pocket. We estimate that each seine sampled 665 $m^2$.

Dungeness crabs caught in each seine were immediately placed in buckets of water from the estuary. A battery-operated air bubbler was added to each bucket to maintain dissolved $O_2$ levels in the water. In the field, immediately after the seine, crabs were measured and sexed. Crab carapace widths (CW) were measured to the nearest millimeter just anterior to the tenth antero-lateral spine using a plastic ruler and/or Vernier caliper. Crabs were sexed by visual examination of their abdominal flaps when possible (narrow/thin for males, wide and rounded for females); sex determination is difficult in early instars. When too many crabs were caught to process in a reasonable amount of time, a minimum of 30 haphazardly selected individuals were handled as above and the remaining individuals were only batch-counted to obtain the total number of individuals caught for density calculations.

Water temperature and salinity from four of the SSNERR system-wide monitoring program (SWMP) water quality stations (**Figure** 1) were downloaded from the NOAA NERR Central Data Management Office (CDMO) website (http://cdmo.baruch.sc.edu/). Water quality stations were assigned to the same four categories as sampling locations: 1. Close to the mouth (Charleston Bridge; Station 1), 2. Middle estuary (Valino Island; Station 2), 3. Upper estuary, Winchester arm (Station 3), and 4. Upper estuary, Sengstacken arm (Station 4). The data collected from these stations were subject to quality assurance/control procedures of the NERRS CDMO prior to being downloaded (*NOAA NERR SWMP*, n.d.) and only data points flagged as "Passed initial QAQC" and "Corrected" were used in analyses. Additionally, a handheld YSI water

quality meter was used to measure water temperature, salinity, pH, and dissolved $O_2$ concentration in the field just prior to the start of each seine.

There were two departures from the above sampling procedure. The first month of the survey (July 2015), crabs were only counted and not measured or sexed, and sampling was limited in 2018, covering only the summer and fall season at two sampling stations (Stations D & F).

*Larval Arrival and Abundance*

Between April and October of each year Dungeness crab megalopae were collected and counted daily using a light trap hung from the floating docks in the Charleston, OR marina (**Figure** 1). The light trap, described in detail by Miller and Shanks (2004), was made by suspending a waterproof 120v LED light strip inside a 19 L water cooler bottle with eight funnel-shaped holes (~ 3 cm I.D.) cut in the side of the bottle. A large hole was cut in the bottom of the bottle to allow the sample to strain through a 256 μm mesh in the cod-end of the trap. Dungeness crab megalopae are positively phototactic and this trap has been used since 1997 to quantify the abundance and timing of larval settlement on this part of the Oregon coast (Miller and Shanks, 2004; Shanks, 2006; Shanks and Roegner, 2007; Shanks et al., 2010; Shanks, 2013).

*Data Management and Analysis*

All raw data were maintained in a Microsoft Access relational database. Data was queried from this database, converted into comma separated files, and read into R (R Core Team, 2018). Data manipulation and plotting was completed using the dplyr and ggplot2 packages in R (Wickham, 2016; Wickham et al., 2018). For each seine, crab density (indv./$m^2$) was calculated as the number of measured crabs ($n_{meas}$) and the number

of crabs which were not measured and only batch-counted ($n_{batch}$) divided by the area ($m^2$) seined and the number of seines completed ($S$) (Equation 1).

*Equation 1*

$$Density = \frac{n_{meas} + n_{batch}}{665m^2 * S}$$

Distinct year classes of crabs and assignment to annual cohorts was accomplished by fitting a finite mixture distribution to crab carapace width data using the R package flexmix (Gruen and Leisch, 2004). This package estimates maximum likelihood parameters of mixed distributions using a version of the expectation-maximization (E-M) algorithm. Mixed distribution model selection was done by minimization of the Bayesian information criterion (BIC). Classes 0+ are used to describe young-of-the-year, 1+ for those in their second year after settlement, and 2+ for those more than two years after settlement. Distinct annual cohorts were obvious for most months, but the boundary between year classes was difficult to discern late in each year when fast growing young of the year approached the size of 1+ crabs. Catches of 1+ crabs became sparse between August and October of each year, as they approached 100-150 mm CW making model fitting difficult for 1+ crabs (**Figure 2**). For months when we caught too few 1+ crabs to model their distribution, we separated year classes at the point where the right tail of 0+ crab distribution approached the x-axis and covered 99.9% of 0+ crabs ( ~ 3.3 SD from mean). Between March and May of 2016 and 2017, we caught five crabs which were > 50 mm CW larger (135 - 156 mm CW) than the average 1+ crabs at this time (50-85 mm CW). Growth models developed for Dungeness crabs indicate the expected molt increment (size increase after molting) for a 50-85 mm CW crab should be between 15-20 mm CW (Chang et al., 2012). Based on this, it is unlikely that these five larger crabs

are newly molted 1+ crabs and we suspect these are the only 2+ crabs caught during this survey.

Size frequency distributions of male and female crabs were compared using a Kolmogorov-Smirnov test. Average annual crab densities and crab size-at-age data were compared using one-way ANOVA (type-III SS). Logistic regression was used to test the relationship between water temperature and salinity in the estuary and the presence/absence of Dungeness crabs in seining samples. If one or more crabs were caught in a seine, they were considered "present". Water temperature and salinity data used in the logistic model were from YSI readings taken at the time of each seine. We set $\alpha = 0.05$ for all tests. Data were log transformed if Levene's test indicated heteroscedasticity.

**Results**

Out of 344 beach seines in the South Slough, 151 resulted in the capture of *M. magister* crabs; hereinafter referred to as crabs. A total of 5,273 crabs were captured with 2,598 of them being measured/sexed and the remaining 2,675 batch-counted. Most of these crabs (91.7 %) were young of the year (0+), followed by 1+ (8.2 %) and 2+ crabs (0.1 %). No crabs were captured in January. More male crabs were caught (51.2%) than females (42.3%), with the remaining (6.5%) undetermined or not recorded. Male and female crabs did not differ in their size frequency distribution (Kolmogorov-Smirnov test, $D = 0.04$, $p = 0.18$) (**Figure 3**).

*Water Conditions*

Water temperature and salinity data from four NERR SWMP monitoring stations in the estuary show clear seasonal, tidal, and estuarine gradient patterns (**Figures 4-7**).

Summer (July-September), the dry season in this area, was characterized by higher salinity at all estuarine zones and warmer water temperature that increased with distance into the estuary. Fall (October – December) and winter (January – March) were characterized by cooler water temperatures and lower salinities. Wide fluctuations of salinity were still apparent in the Spring, at the end of the rainy season, and water temperatures begin to warm towards summer values. The range of temperatures in each zone tended to decrease from the summer into fall and winter. The range of salinities changed more dramatically over this period and as freshwater input during the rainy season (October – April) increased. Water close to the mouth of the estuary reflected its marine origin and was generally cool and salty. The two SWMP stations in the upper estuary (Stations 3 & 4) displayed two tidally-based temperature and salinity regimes in some seasons (Station 3 in the fall, winter, and spring; Station 4 in fall and winter) as indicated by the bimodal distribution of temperature/salinity observations apparent in **Figures 5 & 7**. Stations 3 and 4 are far into the two major arms of the upper estuary and both receive year-round fresh water input from their respective tributaries. As the tide ebbs and floods (two cycles per day), the salinity and temperature fluctuate as the mixture of fresh and marine water changes, leading to the two, dominant, temperature/salinity regimes seen in this part of the estuary.

A comparison of crab densities to water quality conditions recorded with a handheld meter during each seine (**Figure 8**) shows crabs in the summer were captured throughout the mostly high temperatures and salinities observed. In the fall, increased rainfall and lower atmospheric temperatures result in observations of overall lower temperatures and a wider range of salinities, however, we only saw high densities of

crabs in the upper range of salinity (> 20 ppt). Very few crabs were caught during winter and temperature/salinity measurements reflect the expected lower overall temperatures and salinities. In spring, water temperature observations increase and the upper range of salinity increases. Like in fall, we caught higher densities of crabs in the upper range of salinities during spring. In all seasons combined, most crabs were caught between salinities of 20 – 36 ppt and temperatures of 9 - 20 °C.

Logistic regression showed a significant relationship between the presence/absence of Dungeness crabs in our seines and with water temperature ($z = 4.26$, $p < 0.001$) and salinity ($z = 4.57$, $p < 0.001$). For each increase of 1 PSU salinity, the odds of capturing crabs in a seine increased by 12% and for each increase of 1° C in water temperature the odds of capturing crabs in a seine increased by 19% (**Figure 9**). A classification table showed that the logistic model had an overall accuracy of 66% in predicting the presence/absence of crabs.

*Larval Supply*

Light trap samples indicate crab megalopae arrived in the estuary in early spring in all four years (2015-2018). Megalopae were already in the estuary by the time we started to collect light trap samples in 2015 and 2017 so we are unable to determine when they first arrived, but we began catching them on 28 March and 3 April in 2016 and 2018, respectively (**Figure 10**). Patterns of abundance and periodicity in the arrival of crab megalopae into the estuary varied widely between years. The total number of megalopae caught varied by almost three orders of magnitude and ranged from 3,106 (2016) to 2.76 million (2018). Megalopae were caught in distinct, recurring pulses of high abundance. In 2015-2017, these pulses lasted several days and occurred every few

weeks, typical of Shanks' (2013) observations. Most years, megalopae catches decrease to near zero between pulses, but after the first large pulse of 2018 began on 18 April, daily catches did not drop below 1,000 until 17 May (average: 82,000 * $day^{-1}$). Pulses of megalopae were observed as late as mid-October in 2016, but catches fell to zero as early as the end of July in 2018. Late summer catches of megalopae (after 1 August) occurred in all years except 2018. The highest number of late season catches was in 2017 (7,376), with 2015 and 2016 much lower (146 & 75, respectively).

*Spatiotemporal Patterns*

Overall 0 + crab density in summer and fall months was significantly different between years (one-way ANOVA, $F_{3, 337} = 19.91$, $p < 0.001$) (**Figure 11**). A post-hoc (Tukey HSD, $p < 0.05$) test revealed that there were significantly higher 0+ densities in 2018. We saw a massive settlement event in 2018 and crabs in the 0+ year class drove the much higher average density that year. The summer and fall density of 1+ crabs was similar in all years and no 2+ crabs were caught during this period (one-way ANOVA, $F_{3, 337} = 0.19$, $p = 0.90$).

Very few crabs were caught in beach seines between December and April. Of those caught during this time, most were 1+ or 2+ crabs. We began to see 0+ crabs around May or June, one to two months after megalopae were detected in the light trap (**Figure 12**). We caught a few 0+ crabs as small as 7 mm CW, but most were not captured until they were greater than 10 mm CW. The distribution of crab sizes within each year class often appeared as multi-modal mixtures of different cohorts. This was particularly true of 0+ crabs captured later than August, as we began catching many early instar crabs in years when light trap samples indicated late season larval delivery had

occurred (2015, 2016, & 2017). Densities of 1+ crabs in the upper estuary were low throughout each year. The density of 1+ crabs peaked in June in the middle of the estuary and in October closer to the mouth. Average within year crab density was similar in all parts of the estuary except in 2017, when density was very low in the upper estuary (**Figure 13**). Overall crab density decreased throughout the estuary in fall until they were largely absent from seines between December and May of the next year (**Figure 12**).

*Growth of Annual Cohorts*

The size frequency measurements indicated clear patterns of growth (**Figure 14**). By November of each year, about six months after settlement, 0+ crabs attained an average CW of 66.5 mm (2015), 62.3 mm (2016), 67.6 mm (2017), and 51.5 mm (2018). In 2015, 2016 and 2017, these averages included crabs that settled late in the season and were much smaller than 6-month old crabs. We used the mixed distribution models we fit for separating annual cohorts to distinguish between 0+ crabs which likely settled late in the recruitment season. These models suggested that in November of 2015, 2016, and 2017, crabs < 25 mm CW did not come from the dominant 0+ size frequency distribution and were significantly smaller. Excluding these late season settlers from the average size of 0+ crabs showed that after 6 months crabs in 2018 were significantly smaller than those in the other three years (one-way ANOVA, $F_{3, 103} = 7.26$, $p < 0.001$). We were able to follow annual cohorts of crabs into their second year (1+ year class), but they were uncommon in seine samples by December (~ 1.5 years after recruitment). Most of these crabs were between 100 – 160 mm CW by this time. We caught few 2+ crabs. The ones we caught were seen early in the calendar year and were around 150 mm CW, not much larger than they were as 1+ crabs late in the previous year.

## Discussion

Three years (2015-2018) of monthly beach seining in the South Slough estuary has demonstrated the importance of this estuary as nursery habitat for juvenile and sub-adult Dungeness crabs. The young-of-the-year grow rapidly in their first summer and are the appropriate size (> 100 mm CW) by the middle of their second summer (~14 mo. after settlement) to begin reproductive activity (Butler, 1961). Few 2+ crabs were caught during the survey suggesting that almost all 1+ crabs leave the estuary by the end of their second fall season (1.5 years post-settlement).

### *Growth*

Size at age (6 mo.) data reported in the literature for 0+ Dungeness crabs are variable (15 - 90 mm CW) (Wainwright and Armstrong, 1993) and likely depend on local conditions like density, temperature, and food availability. In our study, we observed average growth of 0+ crabs in the middle to upper range of values reported in the literature in all years, even in 2018 when we saw the least growth. By November of their first year (6-7 mo. post-settlement), the average size of 0+ crabs in the South Slough (2015-2017) was 65 mm CW (range: 62.3 – 67.6 mm). The actual average size of crabs that settled six months prior would be slightly higher because very small crabs that arrived as late season settlers are included in these averages. In 2018, the average size at six months was only 52 mm CW (there was no late season settlement this year). Juvenile Dungeness crab growth rates are significantly faster in estuarine systems than for those in the nearshore (Armstrong and Gunderson, 1985; Carrasco et al., 1985). Armstrong and Gunderson (1985) showed 0+ crabs in Grays Harbor grew to 38.3 mm CW six months after settlement (May-October) and during a concurrent study offshore Carrasco (1985)

reported that crabs only reached 15 mm CW by September of the same year (5 mo. post-settlement). Dumbauld (1987) made the same observation in Grays Harbor, where after six months 0+ crabs in the estuary were larger (34 mm CW) than their offshore counterparts (21 mm CW). To our knowledge the largest reported average size at six months is 80.7 mm CW in San Francisco Bay (Tasto, 1983). Previously observed sizes of crabs one year and 1.5 years after settlement (20 – 120 mm CW, 62 - 140 mm CW; respectively) also vary widely between studies (Wainwright and Armstrong, 1993). Crabs caught in this survey were 82 mm CW in May (13 mo. after settlement) and 127 mm CW by October (1.5 years after settlement), in the upper range for both values.

Rapid growth in early stages can be an important adaptation for Dungeness crabs. Observations by Armstrong et al. (1995) that only crabs < 25 mm CW were consumed by a major predator of Dungeness crabs (staghorn sculpin, *Leptocottus armatus,* Girard) suggests there is a size refuge from predation for juvenile crabs. Although, Reilly (1983) saw crabs as large as 150 mm CW in fish stomachs in the Gulf of the Farallones and San Francisco Bay area and believes they were eaten when their exoskeletons were still soft after molting. There are several possible explanations for the rapid growth of Dungeness crabs in their first year in the South Slough. A number of studies have suggested or experimentally demonstrated that warmer temperatures result in faster growth rates (McMillan et al., 1995; Wainwright and Armstrong, 1993). The average summer and fall (when crabs are abundant) temperatures at all stations in the South Slough were 16.5 °C and 11.5 °C, respectively and water in the upper estuary averages 18 °C in the summer (*NOAA NERR SWMP*, n.d.). For comparison, average surface water temperatures recorded at an offshore buoy just north of Coos Bay were 13.7 °C in summer and 12.6 °C

in fall. Considering the water temperature periodically rises above the average, conditions in the estuary are likely at the upper range of thermal tolerance for juvenile Dungeness crabs, particularly in summer, as Sulkin et al. (1996) observed high mortality of juvenile crabs at temperatures > 18 °C. Gutermuth and Armstrong (1989) observed the metabolic rate ($Q_{O_2}$) of 0+ crabs increased with temperatures between 6-14 °C, but did not increase between 14 - 18 °C. This physiological response may allow 0+ crabs to take advantage of warmer estuarine temperatures without excessive metabolic costs (Gutermuth and Armstrong, 1989). Other possible explanations for rapid growth may be food availability and population density. Work by Terwilliger and Dumler (2001) suggests that food availability may be more important than temperature in mediating growth. They observed that crabs under both high and low volume-feeding regimes held in 14 °C water molted less frequently, but ultimately attained larger sizes than crabs held in 21°C water. We do not have any data to present regarding food available to crabs in the South Slough and suggest this as an important step in further investigating the nursery function of this estuary. Iribarne et al. (1994) demonstrated that agonistic interactions and space competition between juvenile Dungeness crabs mediated crab densities and found that consumption rate of food per capita decreased at high densities. We observed similar growth of 0+ crabs in years where summer and fall densities were low (119 – 272 indv./ha; years 2015-2017), but saw a decrease in growth in 2018 when a massive settlement event resulted in an average summer and fall density of 2,017 indv./ha. It is possible that crabs in 2018 grew less due to some combination of reduced food availability and higher densities leading to difficulty finding and handling prey items.

*Larval Supply*

Annual larval supply to the estuary, as measured by catches of megalopae to a light trap, varied by several orders of magnitude. This is not unusual and has been observed by Shanks (2013) over 19 years of sampling at the same location. The catch of megalopae in the light trap in 2018, however, was the highest ever recorded in the 19-year dataset. Most of the megalopae caught this year were captured during a massive, long-lasting pulse at the beginning of the recruitment season, atypical of the patterns in previous years (Shanks, 2013). Shortly after the light trap began catching large numbers of megalopae in 2018 (> 100,000 $day^{-1}$), Thomas (unpublished data) observed several thousand Dungeness megalopae $m^{-2}$ settled onto a mudflat in the Coos Bay estuary, 1-2 km from the mouth of the South Slough. Megalopae were backed into the mud with their eyes and rostrum partially sticking out. Two days later the same mudflat was covered with first instar juveniles backed into the mud in the same manner. Crabs were very abundant (~200-500 $m^{-2}$) in a nearby seagrass (*Zostera marina*) bed on 5 May but by 15 July the density had dropped to ~ 12-15 $m^{-2}$. These observations were limited in scope, but demonstrate that high numbers of newly settled Dungeness crabs occur soon after they are observed in the light trap and that a rapid decrease in density occurs shortly after large settlement events. We do not know the drivers of this rapid decline in density, but other studies point to emigration from areas of high density (Iribarne et al., 1994), cannibalism (Fernández, 1999), and predation by estuarine fish like the staghorn sculpin (Armstrong et al., 1995) as likely factors.

*Spatiotemporal patterns*

Dungeness crabs are highly mobile and are known to undertake ontogenetic migrations (Dumbauld et al., 1993; Stone and O'Clair, 2001) and move between different

habitats in response to tidal cycles and water conditions (Holsman et al., 2006; Curtis and McGaw, 2012; Froehlich et al., 2014). Crab density in the South Slough peaks in summer during the annual settlement season when 0+ crabs are abundant and is very low in winter. Tasto (1983) reported catches of crabs in San Francisco Bay never occurred at salinities below 10.2 PSU and suggested crabs move out of the estuary in response to increased freshwater input. This may be occurring in the South Slough as crabs are largely absent from seine samples by the end of fall after the beginning of the rainy season and remain rare during winter when average salinities are the lowest (17.8 PSU, average of all stations), especially in the upper estuary (8.3 PSU, Station 3; 16.4 PSU, Station 4.) (**Figure**s 4 & 5). It is unknown where the crabs go during this time. It is possible they are in deeper channels within the estuary where our gear was not able to sample. Dumbauld (1987) suggested that crabs bury in the sediment during the winter and are largely unavailable to sampling gear.

We caught very few 2+ crabs during this survey and thus it appears crabs leave the estuary in their second year as they reach reproductive maturity and do not return to the South Slough for a third summer. This is consistent with the observations of Gunderson (1990) that 1+ crabs mostly leave Grays Harbor by the end of their second year, although crabs in the South Slough would be slightly larger when they leave the estuary (~ 25 mm CW bigger) than those in Grays Harbor. We did not sample other locations in the Coos Bay estuary, however, and we do not know if 1+ crabs are making their way to the nearshore or if they are leaving the South Slough to inhabit the main channel in the Coos Bay estuary. Within-year crab densities in the South Slough were similar between the three estuarine zones in this survey except in 2017 when density in

the upper estuary was very low. Between year densities were also similar, except in 2018, when a massive settlement event increased 0+ crab density tenfold. Similar between year densities are surprising given the very different number of megalopae caught each year in a light trap near the mouth of the estuary. This may reflect some level of carrying capacity for the estuary or higher compensating levels of predation in years when megalopae are abundant. We can only speculate about this without more data.

*Limitations*

While thorough in duration, spatial coverage, and sampling interval, this survey has a few shortcomings that make interpretation of the data challenging; particularly with respect to density estimates. At principle issue is the use of a seine net to capture crabs. The study was part of a larger project to replicate the methods of Bottom et al. (1988) and examine how current patterns of fish assemblages within the South Slough compare to this earlier study. Most studies employ some sort of trawl or crab ring to sample crabs and we are unaware of any study which used seining. We do not have data on the capture efficiency of our seine net and do not know if it was biased to certain sizes of crabs. Dungeness crabs are often found buried in the sediment with only their eye stalks and antennules sticking out and it is possible the seine net skipped over buried crabs. Since we cannot estimate capture efficiency of our gear, our density estimates should be considered more of a catch per unit effort than true approximation of actual crab density [Note: this only applies to densities resulting from seines, densities observed by Thomas (unpublished data) on a nearby mudflat were representative of actual densities]. Additionally, the limited seining done in 2018 (only two sites in summer and fall) makes it difficult to compare crab abundance and habitat use with our other survey years.

## Conclusion

Estuaries have been shown to be important nursery grounds for juvenile and sub-adult Dungeness crabs and a large body of work describes the abundance, growth, and habitat use of Dungeness crabs in larger estuaries within their range. Very little is known about how these factors compare in smaller estuaries like the South Slough. Here we have demonstrated that crabs in the South Slough estuary grow rapidly and leave the estuary by the end of their second summer, as they reach sexual maturity. Rapid growth in their first year of life may relieve South Slough crabs of some predation pressure compared to slower growing crabs in other estuaries and those that settle in the nearshore. It is clear from these results that the South Slough estuary serves as important nursery habitat for juvenile and sub-adult Dungeness crabs. Understanding the population dynamics in smaller estuaries like this will help to further develop growth models for young Dungeness crabs, which vary widely among locations, and will help to understand the impact these important consumers have on estuarine ecosystems.

The next chapter explores the trophic ecology of juvenile Dungeness crabs in the South Slough estuary using fatty acids as biomarkers. This experiment represents the initial steps in being able to use fatty acids to infer wild juvenile diets and further our understanding of the important role this species plays in estuarine environments.

# CHAPTER III

# JUVENILE DUNGENESS CRAB (METACARCINUS MAGISTER, DANA) FED MONO-SPECIFIC DIETS ARE DISTINGUISHED BY THEIR FATTY ACID COMPOSITION

This chapter includes previously un-published, co-authored material. Aaron Galloway, PhD and Julie Schram, PhD provided the experimental design of Feeding Assay 1 and supervised REU intern Zade Clark-Henry as he maintained the experiment. Alan Shanks, PhD and Bree Yednock, PhD assisted in the preparation of the manuscript. I was responsible for the experimental design of Feeding Assay 2, lipid extractions, data analysis and writing of the manuscript.

**Abstract**

Juvenile Dungeness crabs are voracious consumers of a wide variety of taxa and their feeding habits can have significant impacts on the community structure in their juvenile habitat. The annual abundance of young-of-the-year (0+) Dungeness crabs fluctuates dramatically and they are periodically observed in high densities in nearshore and estuarine habitats. High densities of juvenile crabs may represent an important, episodic disturbance to food webs in juvenile habitat. We used laboratory feeding assays to investigate the use of fatty acids (FA) as biomarkers in juvenile Dungeness crabs. Additionally, we compared the FA composition of juvenile crabs collected in the field to those fed controlled diets in the laboratory. We found that juvenile crabs fed mono-specific diets rapidly assimilate dietary FA into their tissues and their FA composition is readily distinguished by multivariate analyses. Crabs fed fish, bivalve, and conspecific megalopae grew faster than crabs fed algal-based foods. The ratio of ω3/ω6 FA in crab

lipids was significantly higher in faster growing crabs and may be a useful metric to assess crab condition and diet quality. The FA profiles of juvenile crabs collected in the field were distinct from those fed in the laboratory, but were closest in multivariate space to those fed bivalve diets. Wild crab FA were separated from experimental crabs by elevated levels of bacterial and copepod indicator FA (odd length / branched chain FA & 20:1ω11/20:1ω9, respectively). Wild 0+ crabs were collected during a year of exceptionally high Dungeness crab settlement (2018). This year was characterized by slower 0+ growth than the previous three years when settlement was much lower. The proportion of DHA (22:6ω3) in wild crab lipids was lower than all experimental crabs. Long chain poly-unsaturated FA, like DHA and EPA (20:5ω3), have been implicated as being important for growth in several crustacean species. It is possible slow growing 0+ crabs in 2018 were the result of a limitation in the availability of high-quality foods rich in DHA.

**Introduction**

Dungeness crabs (*Metacarcinus magister*, Dana (1852); formerly *Cancer magister*) are a valuable commercial species and ecologically important predator in the northeast Pacific (Rasmuson, 2013). The annual abundance at the coast of Dungeness crab megalopae fluctuates by a factor of 1000 (Shanks and Roegner, 2007; Shanks et al., 2010) and periodically large settlement events (Galloway et al., 2017) result in high densities (upwards of tens of thousands $m^{-2}$) in nearshore and estuarine habitats on the west coast of Oregon. These massive settlement events may represent significant disturbances to these habitats and are not well understood (Galloway et al., 2017). A disturbance event, in an ecological context, involves biotic or abiotic forces resulting in a

significant departure from some established baseline (Rykiel, 1985). Juvenile Dungeness crabs are voracious consumers potentially feeding on an array of taxa, to include conspecifics (Fernández, 1999), bivalves, fish, polychaetes, shrimp (Gotshall, 1977; Stevens et al., 1982), benthic and epiphytic diatoms (Jensen and Asplen, 1998), and macroalgae (Thomas, pers. obs.). High densities of 0+ crabs (young-of-the-year) may have a serious impact on benthic communities and energy transfer out of juvenile habitat. Estuaries serve as important nursery habitat for juvenile Dungeness crabs (Tasto, 1983; Rooper et al., 2002; Stevens and Armstrong, 1984), where they grow rapidly and eventually migrate to adult habitat in the coastal ocean (Gunderson et al., 1990). The settlement of Dungeness crab megalopae in estuarine systems, and the food habits of juvenile crabs while there, may represent an important link between estuarine and nearshore energy flux as young crabs mature and eventually migrate to the outer coast. Furthermore, density-dependent effects during years of high settlement may regulate annual cohort success and ultimately impact future adult populations (Fernandez et al., 1993; Iribarne et al., 1995, 1994; Shanks, 2013).

Previous studies of juvenile Dungeness crab diets were based on inspection of stomach contents (Gotshall, 1977; Stevens et al., 1982), but these methods can be biased to prey items with easily recognizable hard-parts and may entirely miss important soft-bodied sources of nutrition (Pond and Sargent, 1998), especially in very small crabs. Crabs use their chelae and mandibles to tear food into small pieces during feeding and this could further impede accurate assessment of dietary components. Additionally, gut content analyses can only indicate recently consumed items and do not provide information on the overall diet of an individual. For these reasons, it is important to take

an integrated approach to determining trophic relationships. Biomarkers are measurable biochemical indicators and can be a useful supplement to gut content analyses. Biomarkers are not subject to the same biases, although they do have their own limitations (Dalsgaard et al., 2003; Kelly and Scheibling, 2012).

Fatty acids (FA) have been used as biomarkers in trophic investigations for decades and extensive data are available on the FA profiles of many marine species (Dalsgaard et al., 2003). Prey FA may be incorporated into consumer tissues with little to no modification, providing a direct link between diet and animal tissues (Iverson, 2009). Only plants and algae have been observed to bio-synthesize ω3 and ω6 FA in significant levels, which are critical to the health of animals and must be obtained from their diet (Sargent and Henderson, 1995; Kelly and Scheibling, 2012). As a result, these FA are bio-accumulated into higher trophic levels and can be used to infer trophic positioning (Iverson et al., 2004). Additionally, some invertebrates, like copepods, and bacteria synthesize unique FA which makes it possible to trace the origins of these FA in consumers. Much of the work using FA as biomarkers has been done in the pelagic environment (reviewed by Dalsgaard et al., 2003), where FA are more easily traced from phytoplankton to progressively larger consumers. The use of FA as biomarkers in higher level consumers, such as crabs, is more challenging because mixed diets and a greater diversity of prey makes it impossible to tell if a particular marker FA was ingested when consuming the original producer of the marker or by preying on a predator of this organism. The ability of some benthic invertebrates to significantly modify or preferentially assimilate dietary FA creates another challenge for using FA as trophic markers (Bell and Tocher, 2009). These limitations demonstrate the need to use multiple

markers paired with controlled feeding assays and a library of putative sources of dietary FA when making trophic inferences of benthic consumers (Galloway et al., 2015).

Herein we investigated the diet of wild 0+ juvenile Dungeness crabs in an estuary on the southern Oregon coast using FA analyses and compare the results with two controlled feeding assays of early instar juveniles fed a variety of mono-specific diets. We used multivariate FA signature analysis and a variety of FA ratios and metrics to describe animal condition and propose potential sources of trophic markers. We found that juvenile Dungeness crabs rapidly assimilated dietary FA and when fed singular diets were readily distinguished by their FA profiles depending on their diets. Additionally, we observe that a limitation of an essential ω3 FA may have contributed to poor growth rates of wild crabs during a year (2018) when a massive settlement event resulted in high juvenile densities.

## Materials and Methods

### *Feeding Assays*

We conducted two laboratory feeding assays of juvenile Dungeness crabs fed a variety of monospecific diets. Assays were carried out in two different years (2017 and 2019). The first assay used a taxonomically diverse array of readily available foods to determine if the FA profile of crabs fed different foods would be distinct. These resources were hypothesized as putative foods that crabs may consume in the wild. The second assay investigated if crab FA could be differentiated when fed putative foods collected in the crab's estuarine juvenile habitat contemporaneous to the beginning of their typical settlement season (April - May). The emphasis of feeding assay two was on diets observed as abundant putative sources in the estuary (Thomas, pers. obs.). Both assays

were conducted at the Oregon Institute of Marine Biology (OIMB) in Charleston, OR using crabs caught as megalopae ~ 2 km from the mouth of the Coos Bay estuary using a light trap as described by Miller and Shanks (2004). After metamorphosis, juvenile crabs were haphazardly selected and placed individually in an array of plastic containers partially submerged in an indoor sea table. The plastic containers were supplied with a constant supply of flow-through filtered seawater (FSW) to maintain adequate dissolved $O_2$ levels and experienced a natural photoperiod from the ambient light of nearby windows. Filtered water was used to remove potential food sources that frequently enter the OIMB seawater system. The OIMB seawater system pumps seawater from the mouth of Coos Bay at high tide and thus consists predominantly of coastal seawater. The ambient temperature and salinity of the water ranged from 11 – 14 °C and 31 – 34 PSU in both trials. Crabs were fed a wide taxonomic variety of mono-specific diets *ad libitum* for six weeks. Prior to each feeding, uneaten food and crab excrement was removed from each container. Initial carapace widths (CW) of each crab were measured photogrammetrically using ImageJ software (Schneider et al., 2012) and subsequent measures were taken the same way after each molt cycle. CW was measured as the linear distance between the left and right side of the crab carapace, just anterior to the tenth antero-lateral spines. At the completion of each experiment, crabs were starved for one week to purge their stomach contents before being frozen (-20 °C) and lyophilized. Both feeding assays were conducted in this general manner with any variations discussed below.

*Feeding Assay 1*

Juvenile crabs used in this assay were caught as megalopae over the course of several days at the beginning of June 2017. Megalopae were pooled from several days of collections and held together until metamorphosis (1 – 6 days after collection). After megalopae metamorphosed, juvenile crabs continued to be held in the same container and were maintained on a diet of clam meat (*Silqua patula*, Razor clam) for 2-3 weeks. Beginning on 29 June, 65 crabs (n = 13 per treatment) were haphazardly selected and placed individually in cylindrical plastic containers (500 ml) and supplied with 1 μm FSW. Air was bubbled through a Pasteur pipette into each container to ensure adequate levels of dissolved $O_2$. Crabs were evenly assigned to one of five diet treatments: 1. Algae (*Ulva lactuca*; collected locally), 2. Urchin feces (*Strongylocentrotus purpuratus* fed *U. lactuca* in culture), 3. Bivalve (*Silqua patula*, Razor clam; purchased from Chuck's Seafood in Charleston, OR), 4. Megalopae (*M. magister*; megalopae from light trap), and 5. Fish (*Sebastes melanops*, black rockfish cheek meat; caught near Sunset Bay and donated by recreational fisher). All diets were cut into small pieces (~ 1 mm), except urchin feces which was left as it was, and were stored frozen (- 20 °C) until being fed to crabs.

*Feeding Assay 2*

Crabs used in this assay were caught as megalopae on 25 April 2019. Megalopae were pooled and 48 (n = 8 per treatment) were haphazardly selected and placed into individual 1.5 L rectangular plastic containers supplied with 1.7 L $hr^{-1}$ of 5 μm FSW. Megalopae were allowed to metamorphose and then juvenile crabs were held in these containers with no food subsidy until the beginning of the experiment on 6 May. Crabs were randomly assigned to one of six diet treatments: 1. Bivalve (mostly *Clinocardium*

*nuttali*, some juvenile *Macoma sp.*), 2. Ghost shrimp (*Neotrypaea californiensis*), 3. Mysid shrimp (*Neomysis mercedes*), 4. Megalopae (*M. magister*; megalopae from light trap), 5. Polychaete (*Owenia spp.*), 6. Detritus (upper 1cm of sediment near the mouth of South Slough). All food material was collected between the mouth of the South Slough estuary and the middle estuary around the same time that crab megalopae were captured. Crab foods were frozen while fresh and then lyophilized to prevent FA oxidation. Lyophilized tissue was homogenized into a powder using a Vitamix (Olmstead Falls, OH, USA) blender. This powder was added to a 2% sodium alginate solution and thoroughly mixed. The alginate was boiled to ensure complete dissolution of the alginate salts, but to prevent degradation of dietary lipids, the solution was cooled to below 30 °C before food powder was added. Most diets were mixed at a concentration of 10% w/v, but polychaete and detritus were mixed at 20% w/v because heavy sediments found in these diets resulted in less potential food per unit weight. Food/alginate solution was solidified into 300 mg spheres by syringe injection into aqueous 5% $CaCl_2$. Crab foods were prepared in this way to homogenize all tissues in prey items and because the alginate increases the density of positively buoyant lyophilized material enough so it would sink. Crabs in this assay were maintained on these diets until 20 June (6 weeks of feeding).

*Wild Crab Collections*

We collected juvenile Dungeness crabs from the South Slough estuary and subjected them to the same lipid analyses (explained below) to characterize the overall FA composition of wild juveniles and to compare them to crabs fed known diets, including foods collected from the same habitat where wild crabs were collected following Galloway et al. (2014). Juvenile Dungeness crabs were collected from the field

on two occasions (~ 8 weeks apart, end of July – end of September 2018; hereinafter mid-summer & early fall) from three sites (Crown Point, Valino Island, and the Sengstacken arm of the South Slough) in the South Slough estuary (**Figure 16**). Crown Point and Valino Island are in the marine-dominated middle of the South Slough and the Sengstacken site is in the upper estuary closer to one of the main sources of freshwater input for this estuary. We used a combination of beach seines and snorkeling to collect wild crabs. Juvenile Dungeness crabs were very abundant in 2018 and often large numbers were captured. We haphazardly selected crabs (n= 4 per site, per month) from these samples to be used in analyses. Crabs were held in water from the estuary during transport. In the laboratory, crab CWs were measured using Vernier calipers and they were immediately frozen (-20 °C) and then lyophilized until dry (~ 48 hrs). Dried crabs were stored at -20 °C until lipid extraction.

*Fatty Acid Extraction / Quantification*

Frozen, lyophilized crab tissue was prepared for lipid extraction by homogenization using a stainless-steel mortar and pestle. To begin cell lysis and lipid extraction, an aliquot (~ 10 mg) of this tissue was digested in 2 mL of chloroform for 24 hours. Crabs from both feeding assays were too small to investigate tissue specific lipids, hence, the entire animal was homogenized. Juvenile crabs collected in the wild may have been large enough to separate tissue, but to maintain comparability between wild and laboratory reared crabs, we chose to analyze pooled lipids from all tissue types. Since the wild-caught crabs were not starved to eliminate gut contents, each crab was split into two pieces along the sagittal plane, just to the right of the animal's stomach and the smaller

piece (~ 1/3 of the whole animal) was homogenized and used for analyses. In this way we avoided inclusion of the gut content in the analysis.

Lipid extraction and derivatization was carried out as in Schram et al. (2018). Briefly, lipids were extracted by sonication (10 mins) in a 2:1 chloroform: methanol solution. Cellular debris in this solution was washed by addition of 0.9% NaCl and subsequent vortexing. The lipid extract was separated from the solution by centrifugation and aliquots were removed for gravimetric determination of total lipid concentration (Kainz et al., 2017). The remaining lipid extract was derivatized to fatty acid methyl esters (FAME) by heating at 90 °C for 90 minutes after suspending lipids in toluene and adding 1% methanolic sulfuric acid.

FAME were analyzed by gas (He) chromatography and mass spectrometry (Shimadzu GCMS model QP-2020). The GCMS was fitted with an Agilent (Santa Clara, CA, USA) DB-23 high polarity column (30 m x 0.25 mm x 0.15 μm; L x ID x film). Column temperature was programmed according to Taipale et al. (2016) to ensure adequate separation of chromatogram peaks. Fatty acids were identified by relative retention times and by examination of mass and specific ions in mass spectra (Taipale et al., 2016). Peak identifications were checked against a Nu-Check Prep (Elysian, MN, USA) 566 standard. A known concentration of unmethylated internal standard (19:0) was added to each sample at the beginning of the extraction protocol to verify extraction and methylation efficiency.

*Data Analyses*

Chromatogram peaks of fatty acids were integrated using Shimadzu LabSolutions Insight® software. Peak areas were converted to proportion (% contribution of all

identified fatty acids) and arcsin square root transformed. FA proportion data for FA contributing > 1% of all FA identified were used for multivariate comparisons. Multiple statistical routines in PRIMER v.6 (Clark and Gorley, R., 2006) were used (all based on Euclidean distance) to conduct multivariate comparisons of crab fatty acid profiles. We used non-metric dimensional scaling (nMDS) to visualize crab FA profiles in multivariate space. One-way and two-way PERMANOVA ($\alpha \leq 0.05$, 9999 permutations of raw data, type III sums of squares) were used to compare FA profiles by treatments. Similarity percentage (SIMPER) analysis was used to assess which fatty acids contributed to within-treatment similarity and to add vector overlays of important FA to nMDS plots. We used a variety of metrics to assess the overall condition of crabs. Total lipid (mg $g^{-1}$ dry weight) concentration was used to assess overall contribution of lipids to crab tissue. Ratios of Σω3/ Σω6 FA were used to assess animal health (Sargent et al., 1995; Ahlgren et al., 2009). The ratio of EPA (20:5ω3) / DHA (22:6ω3) in conjunction with the proportion of 16:1ω7 to investigate the contribution of diatoms and dinoflagellates to the diet of crabs (Claustre et al., 1988; Budge and Parrish, 1998). We also compared the sum of bacterial FA markers (odd length and branched chain FA; ΣODD includes 18:1ω7), long chain poly-unsaturated fatty acids (ΣLCPUFA; ARA [20:4ω6], EPA, & DHA), and two 18C essential fatty acids (ΣEFA; LIN [18:2ω6] & ALA [18:3ω3]). Additionally, we compared the proportion of copepod indicator FA (20:1, 22:1) in crab tissues. Total lipid, condition metrics, and summations of FA groups were compared using ANOVA in R and visualized using the ggplot2 package (Wickham, 2016). Tukey's HSD was used to conduct post-hoc testing.

## Results

*Crab Size / Growth*

Crabs in Feeding Assay 1 were collected before the experimental period and were all maintained on a diet of razor clam meat until the beginning of the assay. They were second instar juveniles (avg. 8.8 mm CW) at the start of the experiment, having molted once, and likely assimilated some of the lipids from the clam meat into their tissues. At the end of the six-week experiment, all surviving crabs had completed an additional molt, with some molting a second time. Crab survival in Feeding Assay 1 was high (90 %). Survival was 77% in the razor clam treatment, 85% in the fish, 92% in the megalopae, and 100% in the remaining treatments. The average size increase between 2$^{nd}$ and 3$^{rd}$ instar was 3.2 mm CW (± SE 0.06 mm) and the average increase between 3$^{rd}$ and 4$^{th}$ instar was 4.2 mm CW (± SE 0.1 mm). Crabs fed algae and algae-derived urchin feces only molted once and finished at a smaller size (12.0 ± SE 0.2 mm CW) than crabs fed the other three diets (15.7 ± SE 0.5 mm CW) (**Figure 17, Panel A**).

In Feeding Assay 2, crabs were not fed prior to the beginning of the experiment and began as 1$^{st}$ instar juveniles. Only four crabs molted during the six-week experimental period (3 fed bivalve meat, 1 fed megalopae) increasing an average of 2.4 mm CW (± SE 0.2 mm) from 1$^{st}$ to 2$^{nd}$ instar (**Figure 17, Panel B**). No crabs fed the detritus or polychaete diets survived and were unavailable for FA analyses. Survival in the remaining treatments was higher (78%).

Wild crabs caught in the South Slough estuary in mid-summer of 2018 (end of July) were mostly 5$^{th}$ and 6$^{th}$ instars (20 – 30 mm CW). Crabs caught at the same

locations in early fall (end of September) were mostly 7$^{th}$ instars (38 mm CW) with one outlier 9$^{th}$ instar (55 mm CW) caught at Valino Island (**Figure 18**).

*Crab Condition / Total Lipids*

Total tissue lipid content (mg g$^{-1}$ dry weight) of crabs fed different diets was significantly different (one-way ANOVA, F= 3.574, df = 4, $p < 0.05$). Crabs fed fish had significantly higher total lipid content than those fed algae or algal derived urchin feces (Tukey HSD, $p < 0.05$) (**Figure 19**). Total lipid content of wild-caught crabs at three locations in the South Slough did not differ by location or season caught and no interaction between these variables was found (two-way ANOVA, F= 1.342, df= 5, $p > 0.05$). We do not have total lipid concentration data for Feeding Assay 2.

Wild crabs and crabs fed bivalve diets, urchin feces, and ghost shrimp had high proportions of ΣODD (all odd chain length and branch chain FA; also includes 18:1ω7) compared to other diets (one-way ANOVA, $F_{9, 61}$= 10.0, $p < 0.001$, Tukey HSD) (**Figure 20, panel A**). This difference was mainly driven by i17:0 and a17:0 (**Tables 4-6**). Wild crabs had the lowest total ΣLCPUFAs (ARA, EPA, & DHA) and were significantly lower than crabs fed bivalve (cockle), mysids, megalopae, and ghost shrimp (one-way ANOVA, $F_{9, 61}$= 7.0, $p < 0.001$, Tukey HSD) (**Figure 20, panel B**). The lack of ΣLCPUFA in wild crabs was the result of much lower proportion of DHA in these crabs (**Tables 4-6**). Crabs fed algae and urchin feces had much higher proportion of LIN + ALA than other crabs (one-way ANOVA, $F_{9, 61}$= 74.6, $p < 0.001$, Tukey HSD) (**Figure 20, panel C**). The ω3/ω6 ratio of crabs fed algae and urchin feces was similar to those fed megalopae in Assay 1, but was significantly lower than wild crabs and those fed all other diets (one-way ANOVA, F= 13.74, df= 9, $p < 0.001$, Tukey HSD) (**Figure 21**). Crabs fed

fish had the highest ω3/ω6 ratio and were significantly higher than wild crabs (Tukey HSD, $p < 0.05$). The proportion of 16:1ω7 was significantly different between diets (ANOVA, $F_{9, 61} = 4.11$, $p < 0.001$), but a pairwise comparison showed quite a bit of overlap in similarity and only crabs fed mysids had a lower proportion of 16:1ω7 than wild crabs (Tukey HSD, $p < 0.05$) (**Figure 22**). The total proportion of 20:1ω9 + 20:1ω11 varied significantly by diet (ANOVA, $F = 5.01$, $df = 9$, $p < 0.001$, Tukey HSD). Wild crabs had similar proportions of 20:1 + 22:1 mono-unsaturated fatty acids (MUFA) to crabs fed bivalves and megalopae, but were significantly higher than all other diets (**Figure 23, panel A**). A comparison of the ratio of 20:1ω11 / Σ 20:1 + 22:1 between diets showed that 20:1ω11 contributed a greater proportion to the FA of wild crabs (ANOVA, $F = 9.464$, $df = 9$, $p < 0.001$, Tukey HSD) (**Figure 23, panel B**).

*Multi-variate FA Comparison*

A total of 42 unique fatty acids were identified in crab lipids, 24 of which contributed greater than 1% to all FA identified. When visualized in multivariate space (nMDS), FA profiles of crabs in both feeding assays were visually separated by their diets (**Figure 24**). All FA profiles of crabs fed different diets from both assays were significantly different from each other (one-way PERMANOVA; **Table 1**). There was even visual separation between similar diets provided in each assay like megalopae (collected in different years) and bivalve (different taxonomic groups). A SIMPER analysis was used to determine which FA contributed > 5% to similarity within diets. Crabs fed algae were distinguished by 18:1ω9 and 22:5ω3 and crabs fed fish showed a high similarity between 16:1n7, 20:1ω9, and 20:5ω3. In ghost shrimp and mysid fed crabs, 20:1ω11 was an important component FA and 20:1ω9 contributed to a large

percentage of the similarity between crabs fed megalopae and bivalve meat. Even though the overall FA profiles of crabs fed both bivalve diets were different, i17:0 and a17:0 contributed a high percentage to the similarity between these crabs (8.9% & 10.7%, respectively).

Multivariate visualization of wild-caught crab FA profiles showed much less visual separation than crabs fed mono-specific diets in feeding assays (**Figure 25**). A two-way PERMANOVA (**Table 2**) comparing wild-caught crab FA profiles between location and season caught showed a significant difference between locations, but not seasons. There was a significant interaction between location and season, however, and a pairwise comparison (with Monte Carlo permutations) showed that FA profiles of crabs caught at different locations in mid-summer were not significantly different, but were significantly different by location in early fall (two-way PERMANOVA; P (MC) > 0.05).

When FA profiles of all crabs analyzed (wild-caught and experimental) were visualized in multivariate space, there was clear separation between wild and laboratory-fed crabs (**Figure 26**). Experimental crabs fed bivalve diets were closest in multivariate space to wild crabs, but FA profiles of all experimental crabs were significantly different from those captured in the wild (one-way PERMANOVA; **Table 3**).

*Nutritional quality of foods*

FA analyses of the foods fed to crabs in Feeding Assay 2 demonstrated that the detritus and polychaete treatments contained a very small proportion of PUFA, with almost no LCPUFA in detritus (**Table 7**). These foods were analyzed with too little replication (n = 2) to conduct further analyses, but it is clear from these data and the 100% mortality of crabs fed these diets that the FA composition of these foods was not

sufficient to maintain crabs. In Feeding Assay 2, crabs fed megalopae, ghost shrimp, mysid shrimp, and bivalve would immediately begin feeding on food pellets when new ones were added to their containers. In comparison, crabs fed on the detritus and polychaete pellets would immediately investigate newly added food, but generally rejected them and were not observed consuming large quantities of this material.

**Discussion**

The results of our laboratory feeding assays have shown that early instar Dungeness crabs rapidly assimilate dietary FA in their tissues and can be distinguished by their FA profiles when fed mono-specific diets. Juvenile crabs can be maintained on a variety of foods, but algal-based diets caused reduced growth. Crabs fed unmodified tissue of bivalve, fish, and megalopae (*M. magister*) grew faster and molted more frequently than those fed other diets. The ω3/ω6 ratio of crabs fed diets that increased growth was significantly higher than diets which resulted in slower growth and suggests that this metric could be useful in determining food quality for these crabs. The FA profiles of all crabs fed different diets were significantly different after six weeks whether they molted during this time or not. Crabs in Feeding Assay 1 were all maintained on a single diet of razor clam meat from 1$^{st}$ to 2$^{nd}$ instar and then fed different diets between 2$^{nd}$ and 3$^{rd}$ instar. This demonstrates that a shift in juvenile diet can result in significant modification of crab lipids within one intermolt period.

Understanding the trophic ecology of juvenile Dungeness crabs is an important component to understanding their population dynamics. Studies of adult Dungeness crab populations demonstrate wide fluctuations in overall abundance on multi-year timescales (Pauley et al., 1989; Shanks and Roegner, 2007). Commercial landings have been used as

a proxy for adult population size and they are highly correlated with indices of megalopal abundance at the coast (Shanks and Roegner, 2007). Annual catch of Dungeness crab megalopae to a light trap in Coos Bay has varied by several orders of magnitude (Shanks, 2013) and periodically (perhaps regularly) results in high densities of juvenile crabs in nearshore (Galloway et al., 2017) and estuarine environments (Thomas, pers. obs.). Juvenile Dungeness crabs are voracious consumers of a diverse array of foods and, especially in high densities, they may have a significant influence on benthic community structures. A lack of quality food in years of high settlement may also impact annual cohort success, and affect future recruitment into adult populations.

It is unknown whether the high mortality of crabs fed polychaete and detritus in Feeding Assay 2 was the result of them not consuming enough food and starving or if the foods did not contain the proper nutrients for survival. The polychaete treatment is likely not representative of crabs feeding directly on polychaetes as there was relatively little animal tissue from the thin-bodied owenids in the prepared pellets. Pellets made from these worms were largely sediment from worm tubes and may have been more similar to the detritus treatment than representative of worm tissue. Crabs in Feeding Assay 2 did not grow as much as those in the first assay, even those fed similar foods. We believe this may be a result of using alginate pelletized food in Assay 2 as opposed to feeding crabs whole tissue in Assay 1. There was overall less food per unit volume in pelletized food and this may have prevented crabs fed pellets from gaining the same level of nutrition given similar feeding effort. The way crab foods were prepared was modified by pelletizing them with alginate to allow a greater variety of food choices, but this may have prevented crabs from eating as much animal tissue as in unprocessed diets.

Moreover, crabs in Feeding Assay 1 had an initial high-quality diet (bivalve meat) and this may indicate the importance of a head-start for juvenile crabs.

The FA profiles of wild-caught juvenile Dungeness crabs in the South Slough estuary were dissimilar from those maintained in feeding assays, even those fed putative foods from their habitat. When FA contributing > 1% to all FA identified were visualized in multivariate space, wild crabs were most adjacent to those fed bivalves in the laboratory but greater proportions of bacterial indicator FA (mostly i17:0 and a17:0) and copepod indicator FA (20:1ω9 + 20:1ω11) in wild crabs contributed to their separation from experimental crabs. The FA profiles of crabs collected at three different locations within the estuary did not differ in mid-summer (end of July) of 2018, but by early fall (end of September) they were all significantly different. FA contributing the greatest to the differences between the two time periods were i17:0, a17:0, 20:1ω11, and 20:5ω3. Based on their size at capture, crabs at sampling locations underwent 1-2 molts between collections. The difference in FA composition may be due to seasonal or ontogenetic shifts in their diets. Overall, the proportion of DHA (22:6ω3) in wild crabs was lower than all experimental crabs except those fed algae and algal-derived urchin feces. Crabs fed these diets grew slower and molted less frequently. Wild 0+ crabs in the South Slough grew much slower in 2018 when a massive settlement event resulted in high juvenile densities compared to the previous three years when crab densities were much lower (Thomas, unpublished data; Chapter 1 of this thesis).

DHA and EPA have been shown to be important for growth in a variety of crustacean species (Xu et al., 1993; Merican and Shim, 1996; Suprayudi et al., 2004; Sui et al., 2007). It is possible that reduced DHA in juvenile crabs played a role in slower

growth rates observed in 2018. To our knowledge there is no other FA information available in the literature on juvenile Dungeness crab FA. The only reference we found was Allen (1971), who looked at the FA of adult Dungeness and found that adult crabs (n= 8, 4 from each sex) collected close to the entrance to Humboldt Bay, CA in 1969 contained an average of 12 % (no error given) DHA. While FA information is unavailable for juvenile Dungeness, there are several studies which document the DHA content of other crab species. For example, Wu et al. (2007) found wild Chinese mitten crabs (*Eriocheir sinensis*) to contain 10.5% DHA (± 0.6 SE %). Copeman et al. (2012) found early instar red king crab (*Paralithodes camtschaticus*) tissues in Alaska contained 14.75% (± 0.13 SE %) DHA. Wild crabs collected in this experiment contained an average of 8.7% (± SE 0.3 %) and the average proportion of DHA in experimental crabs not fed algae or urchin feces was 13.5% (± SE 0.3 %). Reduced access to high-quality food due to density-dependent effects during a year of massive settlement (2018) may have resulted in the low proportion of DHA observed in wild crab tissues in this study (Thomas et al., unpublished data, Chapter 1 of this thesis).

This study has laid the groundwork for future trophic investigations of juvenile Dungeness crabs using FA. Juvenile crab lipids are influenced by their diet and changes in feeding behaviors of early instars are detectable within a few weeks. Future work should investigate how crabs selectively retain and modify fatty acids using additional controlled feeding assays and should undertake more widescale FA analyses of putative foods in juvenile habitat. With information about how crabs manipulate the FA from their diets and a FA resource library of potential prey items, the analysis of wild-caught crab lipids may be more useful for elucidating juvenile crab trophic ecology.

# CHAPTER IV

# CONCLUSIONS

This study has demonstrated the importance of smaller estuarine systems, like the South Slough National Estuarine Research Reserve (SSNERR), in the early life stages of juvenile Dungeness crabs. Young-of-the year Dungeness crabs grew rapidly in their first year post-settlement and then became rare in our samples by fall of their second year (~1.5 years post-settlement). It is unknown if these crabs have left the estuary or are otherwise unavailable to our sampling. Further investigation should be done to determine if these crabs are actively migrating out of the estuary at this stage. High annual catches of larvae in a light trap near the mouth of the estuary occur when high densities of juveniles are observed in the estuary, indicating the light trap may serve as an index of larval settlement. Crab size at six months post-settlement was significantly smaller in a year (2018) when crabs were observed in high densities and this may have been related to a limitation on access to high quality food sources. We observed a relatively low proportion of DHA, an important ω3 fatty acid (FA), in wild crabs in 2018 when compared to crabs fed putative food sources in the lab suggesting wild crab diets may have been limited in this FA.

This study has laid the groundwork for future investigations of the trophic ecology of wild juvenile crabs using FA as biomarkers as we found that juvenile crabs rapidly assimilate the FA of their diets and those fed different foods can be distinguished from each other with multivariate analyses. Understanding what juvenile crabs are eating in the wild will help to understand how massive settlement events might act as periodic disturbance events within settlement habitats and will help to determine how density-

dependent effects in dense aggregations of juveniles may impact their recruitment success into the adult population.

# APPENDIX A: FIGURE LEGENDS

**Figure 1** – Map of sampling stations, water quality stations, and light trap location in the South Slough estuary, Coos Bay, OR.

**Figure 2 –** Example mixed distribution models from the flexdist package in R. Left panel shows a simple fit where both cohorts (0+ red, 1+ blue) have enough samples for the distribution of both cohorts to be modeled accurately. Right panel shows a month when not enough 1+ crabs were captured to model their distribution accurately. When the 1+ cohort was not able to be modeled, cohorts were separated where the right tail of the 0+ distribution approached the x-axis.

**Figure 3** – Comparison of size frequency distribution between sexes of all Dungeness crabs caught and sexed during the survey. Unknown crabs were either too small to determine sex or sex was not documented while in the field.

**Figure 4** – Comparison of annual and seasonal water temperature (°C) and salinity (PSU) regimes from water quality Station 1 in the South Slough estuary, Coos Bay, OR (*NOAA NERR SWMP*, n.d.). Station 1 is close to the mouth of the estuary and nearest to sampling stations A and B. The rainy season in this area begins in fall and lasts until late winter / early spring. Note the increasing number of low salinity observations between fall and winter. The reverse pattern is apparent between spring and summer. There is very low variability in salinity at this station during the summer dry season.

**Figure 5** – Comparison of annual and seasonal water temperature (°C) and salinity (PSU) regimes from water quality Station 2 in the South Slough estuary, Coos Bay, OR (*NOAA NERR SWMP*, n.d.). Station 2 is near the middle of the estuary and closest to sampling stations C and D. The rainy season in this area begins in fall and lasts until late winter / early spring. Note the increasing number of low salinity observations between fall and winter. The reverse pattern is apparent between spring and summer. In all seasons, more low salinity observations at this station than near the mouth of the estuary suggest greater influence of the riverine upper estuary in this part of South Slough.

**Figure 6** – Comparison of annual and seasonal water temperature (°C) and salinity (PSU) regimes from water quality Station 3 in the South Slough estuary, Coos Bay, OR (*NOAA NERR SWMP*, n.d.). Stations 3 is in the upper estuary, in the Winchester arm of the South Slough. Station 3 is closest to sampling station E, but is farther up the estuary. The rainy season in this area begins in fall and lasts until late winter / early spring. Note the bimodal distribution of salinities in the fall, winter, and spring resulting from tide-mediated shifts between freshwater and more marine water from the lower estuary.

**Figure 7** – Comparison of annual and seasonal water temperature (°C) and salinity (PSU) regimes from water quality Station 4 in the South Slough estuary, Coos Bay, OR (*NOAA NERR SWMP*, n.d.). Stations 4 is in the upper estuary, in the Sengstacken arm of the South Slough. Station 4 is closest to sampling station F. The rainy season in this area begins in fall and lasts until late winter / early spring. Note the bimodal distribution of

salinities in winter resulting from tide-mediated shifts between freshwater and more marine water from the lower estuary.

**Figure 8 -** Density of Dungeness crabs captured in beach seines compared to water temperature and salinity measurements taken by handheld YSI meter before each seine. The area of each point is the square root transformed number of individuals caught per hectare. A transformation was applied to the data so samples with extremely high density did not cover the majority of other samples. Smallest points show where a seine occurred, but no crabs were caught.

**Figure 9 –** Results of logistic regression on crab presence/absence in seine samples as a function of water temperature and salinity. Black points indicate presence (1.00) or absence (0.00) at a given salinity. Colored lines are probabilities predicted by model at different water temperatures indicated by text near each line.

**Figure 10 -** Comparison of daily abundance of Dungeness crab megalopae caught in a light trap in the Charleston, OR marina near the mouth of the South Slough estuary. Julian day 90 is the beginning of April. Number in upper right of each plot is total megalopae caught that year.

**Figure 11 –** Comparison of average annual crab density (error bars are SE) for seines conducted in summer and fall months for each year class. Letters are results from Tukey HSD post-hoc test.

**Figure 12 –** Average monthly crab density (error bars are SE) all years combined by estuarine zone [near mouth (Stations A & B), middle estuary (Stations C & D), upper estuary (Stations E & F)].

**Figure 13 –** Average (error bars are SE) annual summer and fall crab densities by estuarine zone [near mouth (Stations A & B), middle estuary (Stations C & D), upper estuary (Stations E & F)]. No samples were taken near the mouth of the estuary in 2018.

**Figure 14 -** Carapace widths over time of Dungeness crabs captured in beach seines at all sampling stations. Each point represents one crab. Color is year in which each crab initially settled based upon year class assignments from Figure 9.

**Figure 15 –** Comparison of carapace widths of 0+ crabs captured in seines in the South Slough after excluding late season settlers from the analysis based on the results of mixed distribution models.

**Figure 16** – Map of sampling locations in the South Slough estuary, Coos Bay, OR.

**Figure 17** – Comparison of initial and final carapace widths for experimental juvenile Dungeness crabs in Feeding Assay 1 (Panel A, 2017) and Feeding Assay 2 (Panel B, 2019) after six weeks. Bars are mean, boxes are interquartile range, whiskers are range (excluding outliers), and points are outliers. Diet treatment codes are: Algae = *Ulva sp.*, BV1 = razor clam, BV2 = cockle, Feces = purple urchin feces, Fish = black rockfish

cheek meat, Meg1 = megalopae caught in 2017, Meg2 = megalopae caught in 2019, Mysid = *Neomysis mercedes*, Neo = *Neotrypaea sp., Polych = Owenia sp.*

**Figure 18 –** Comparison of wild caught juvenile Dungeness crab (n = 4 per site/season) carapace widths by location in the South Slough caught ~ 8 weeks apart (mid-summer is end of July 2018, early fall is end of September 2018). Bars are mean, boxes are interquartile range, whiskers are range (excluding outliers), and points are outliers.

**Figure 19 –** Total lipid concentration (error bars are SE) of juvenile Dungeness crabs fed mono-specific diets in Feeding Assay 1 (Panel A) and of wild crabs from three locations in the South Slough estuary, Coos Bay, OR. Mid-summer season is end of July 2018, early fall season is end of September 2018. Panel A: One-way ANOVA, $F_{4, 20} = 3.4$, $p = 0.02$. Panel B: two-way ANOVA; Location: $F_{2, 20} = 2.22$, $p = 0.13$; Season: $F_{1, 20} = 0.32$, $p = 0.57$. Letters are post-hoc (Tukey HSD) test results.

**Figure 20 –** Percentage (error bars are SE) of bacterial FA (odd chain length, branched chain, and 18:1ω7; **Panel A**), long-chain poly-unsaturated fatty acids (ARA, EPA, & DHA; **Panel B**), and LIN + ALA (**Panel C**) in juvenile Dungeness crabs fed monospecific diets (Assay 1 & Assay 2) and juvenile crabs caught in the South Slough estuary (Wild). Diet treatment codes are: Algae = *Ulva sp.*, BV1 = razor clam, BV2 = cockle, Feces = purple urchin feces, Fish = black rockfish cheek meat, Meg1 = megalopae caught in 2017, Meg2 = megalopae caught in 2019, Mysid = *Neomysis mercedes*, Neo = *Neotrypaea sp., Polych = Owenia sp.*, Wild = wild-caught crabs.

**Figure 21 –** Ratio (error bars are SE) ω3 (20:3, 20:4, 20:5, 22:5, & 22:6) to ω6 (18:2, 20:2, 20:3, 20:4, & 22:4) FA in juvenile Dungeness crabs fed monospecific diets (Assay 1 & Assay 2) and juvenile crabs caught in the South Slough estuary (Wild). Diet treatment codes are: Algae = *Ulva sp.*, BV1 = razor clam, BV2 = cockle, Feces = purple urchin feces, Fish = black rockfish cheek meat, Meg1 = megalopae caught in 2017, Meg2 = megalopae caught in 2019, Mysid = *Neomysis*. One-way ANOVA, $F_{9, 61} = 10.6$, $p < 0.001$. Letters are post-hoc (Tukey HSD) test results.

**Figure 22 –** Proportion (error bars are SE) of 16:1ω7 in juvenile Dungeness crabs fed monospecific diets (Assay 1 & Assay 2) and juvenile crabs caught in the South Slough estuary (Wild). Diet treatment codes are: Algae = *Ulva sp.*, BV1 = razor clam, BV2 = cockle, Feces = purple urchin feces, Fish = black rockfish cheek meat, Meg1 = megalopae caught in 2017, Meg2 = megalopae caught in 2019, Mysid = *Neomysisi*. One-way ANOVA, $F_{9, 61} = 4.1$, $p < 0.001$. Letters are post-hoc (Tukey HSD) test results.

**Figure 23 –** Sum (error bars are SE) of 20:1 + 22:1 (**Panel A**) and ratio (error bars are SE) of 20:1ω11 / Σ 20:1 + 22:1 (**Panel B**) FA in juvenile Dungeness crabs fed monospecific diets (Assay 1 & Assay 2) and juvenile crabs caught in the South Slough estuary (Wild). Diet treatment codes are: Algae = *Ulva sp.*, BV1 = razor clam, BV2 = cockle, Feces = purple urchin feces, Fish = black rockfish cheek meat, Meg1 = megalopae caught in 2017, Meg2 = megalopae caught in 2019, Mysid = *Neomysis*. Top:

one-way ANOVA, $F_{9, 61} = 4.4$, $p < 0.001$. Bottom: one-way ANOVA, $F_{9, 61} = 9.9$ , $p < 0.001$. Letters are post-hoc (Tukey HSD) test results.

# APPENDIX B: FIGURES

Figure 1

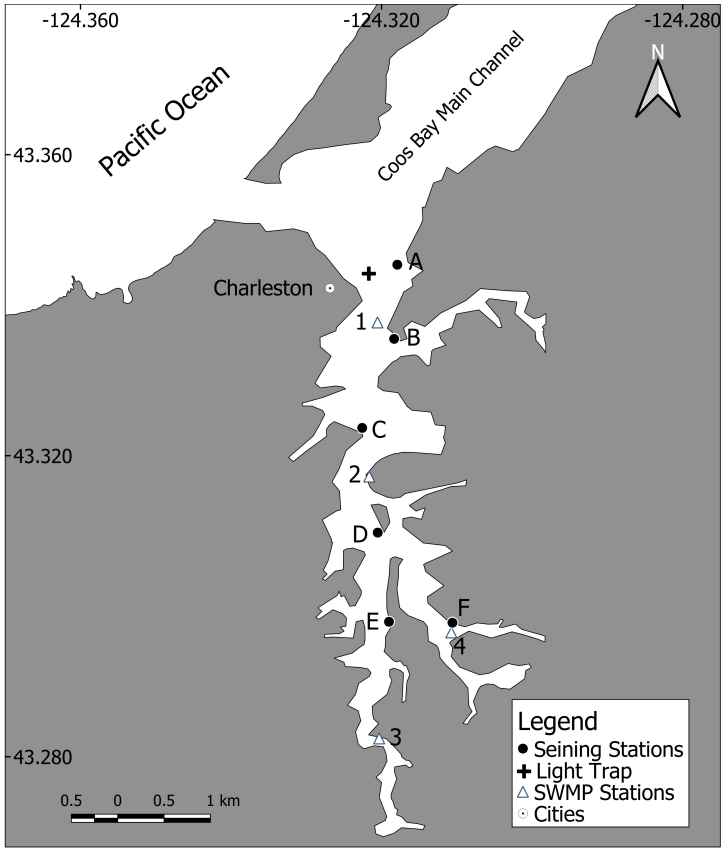


Figure 2

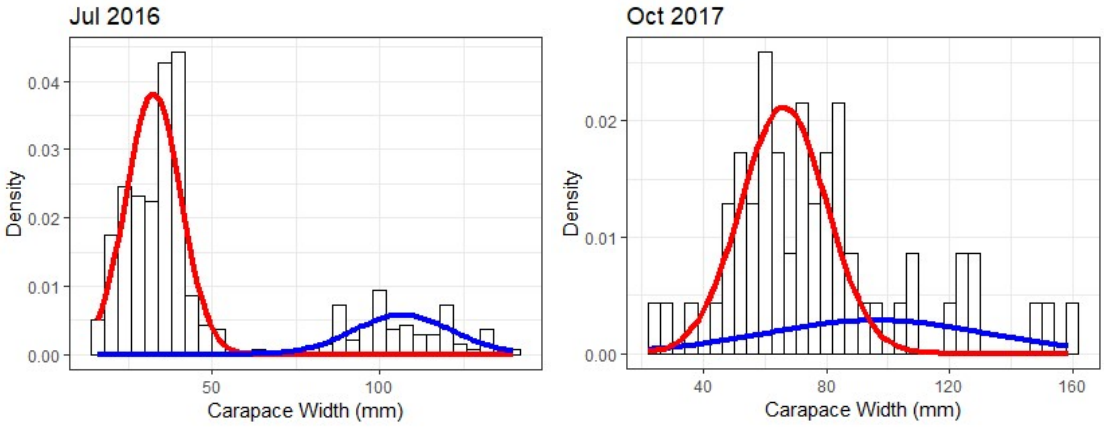

Jul 2016
Density
0.04
0.03
0.02
0.01
0.00
50
100
Carapace Width (mm)
Oct 2017
Density
0.02
0.01
0.00
40
80
120
160
Carapace Width (mm)

Figure 3

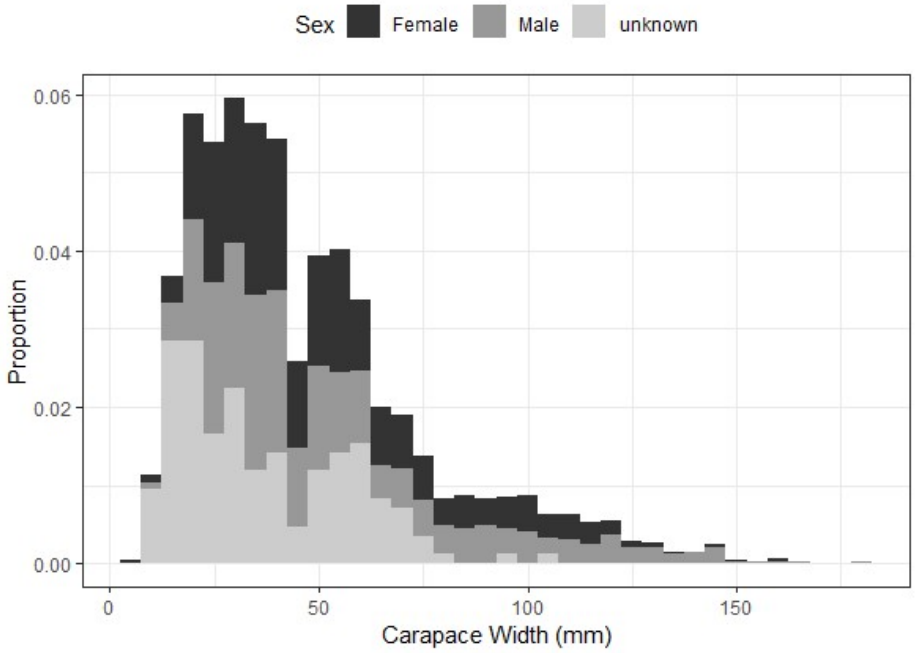

Sex
Female
Male
unknown
0.06
0.04
0.02
0.00
Proportion
0
50
100
150
Carapace Width (mm)

Figure 4

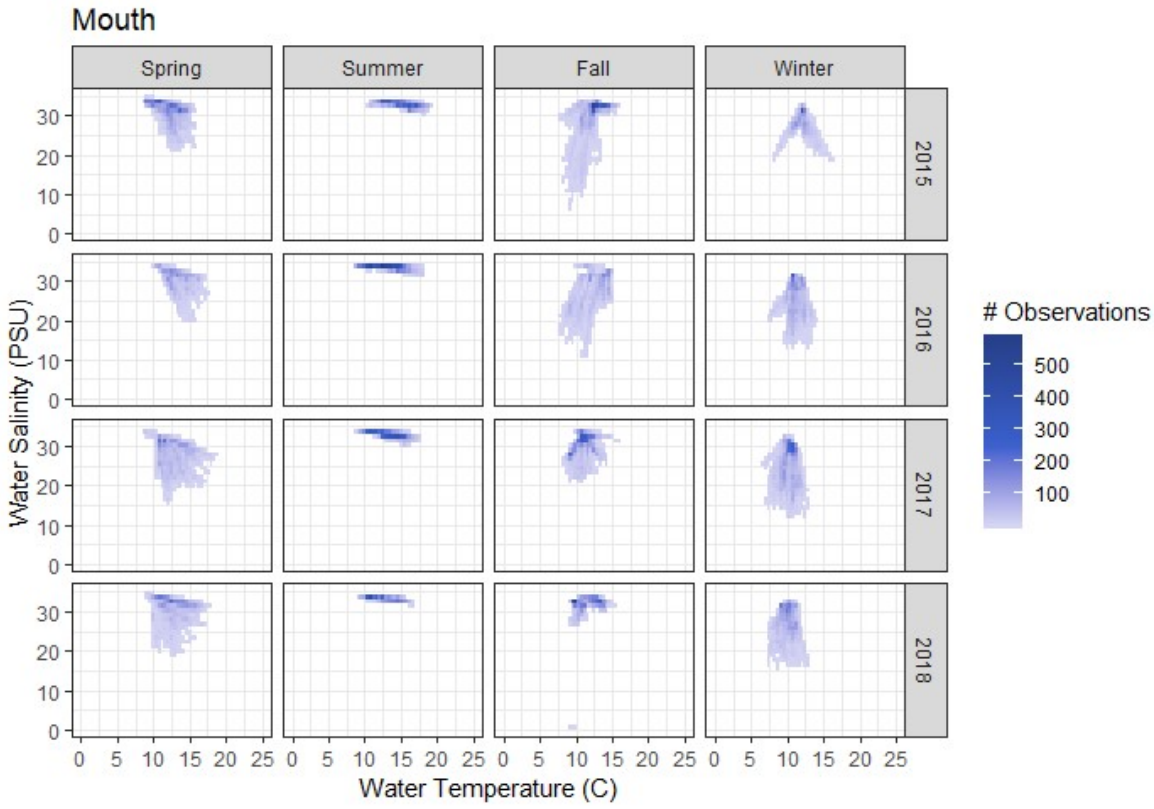

Mouth
Spring
Summer
Fall
Winter
2015
2016
2017
2018
Water Salinity (PSU)
Water Temperature (C)
# Observations
500
400
300
200
100

Figure 5

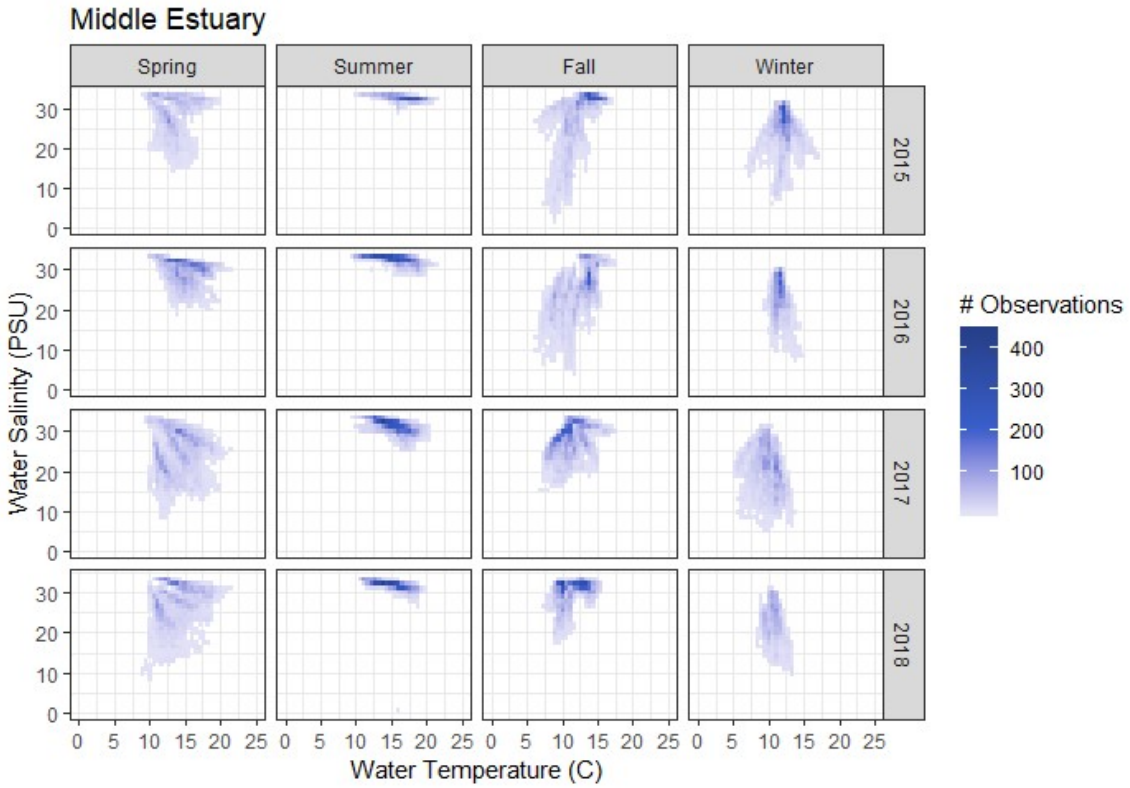

Middle Estuary
Spring
Summer
Fall
Winter
2015
2016
2017
2018
Water Salinity (PSU)
Water Temperature (C)
# Observations
400
300
200
100

Figure 6

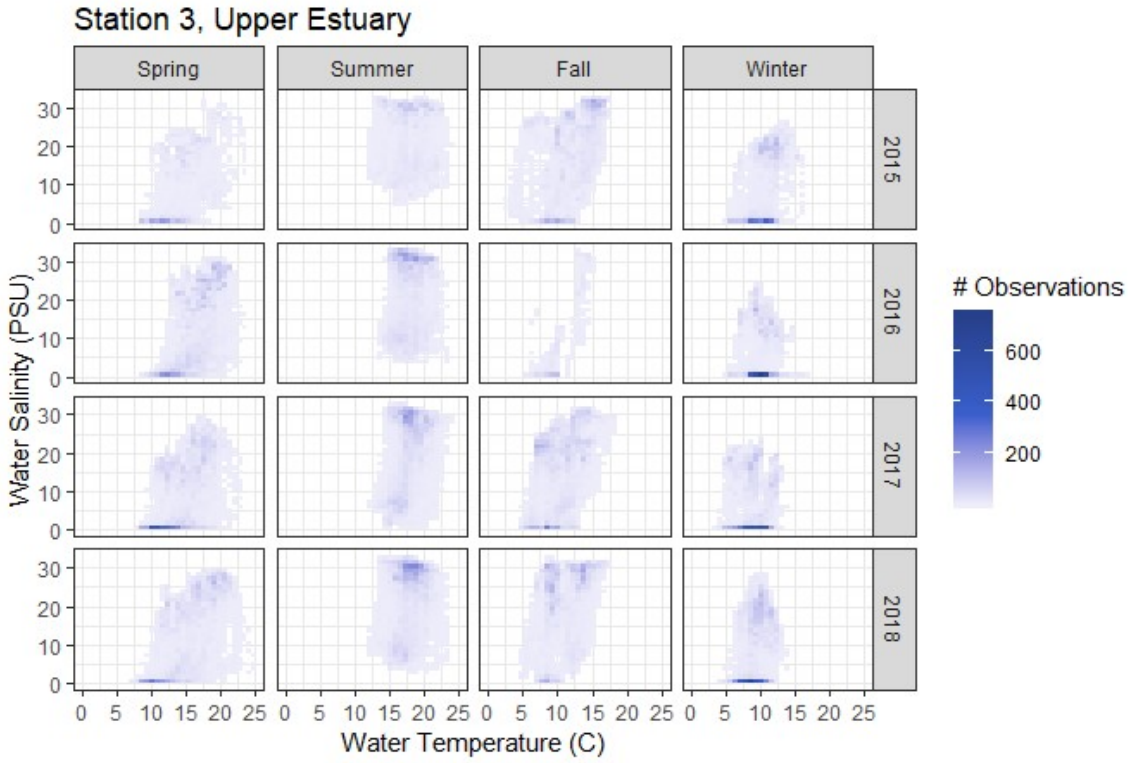

Station 3, Upper Estuary
Spring
Summer
Fall
Winter
2015
2016
2017
2018
Water Salinity (PSU)
Water Temperature (C)
# Observations
600
400
200

Figure 7

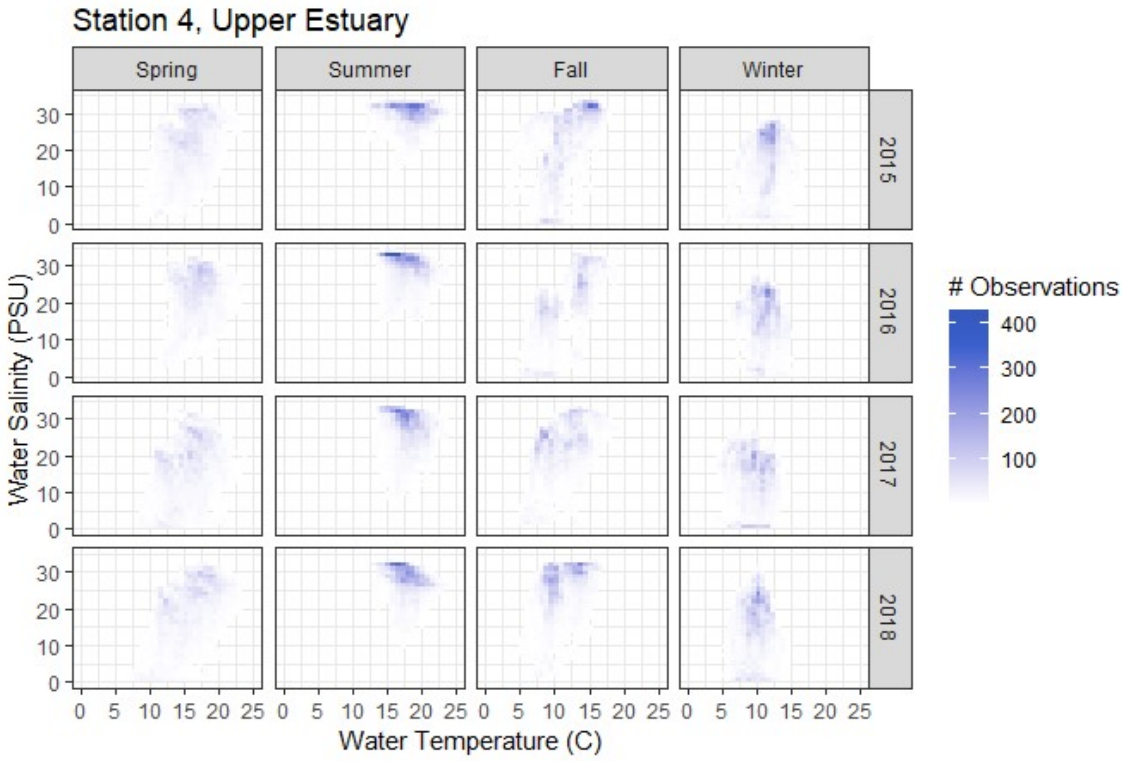

Station 4, Upper Estuary
Spring
Summer
Fall
Winter
2015
2016
2017
2018
Water Salinity (PSU)
Water Temperature (C)
# Observations
400
300
200
100

Figure 8

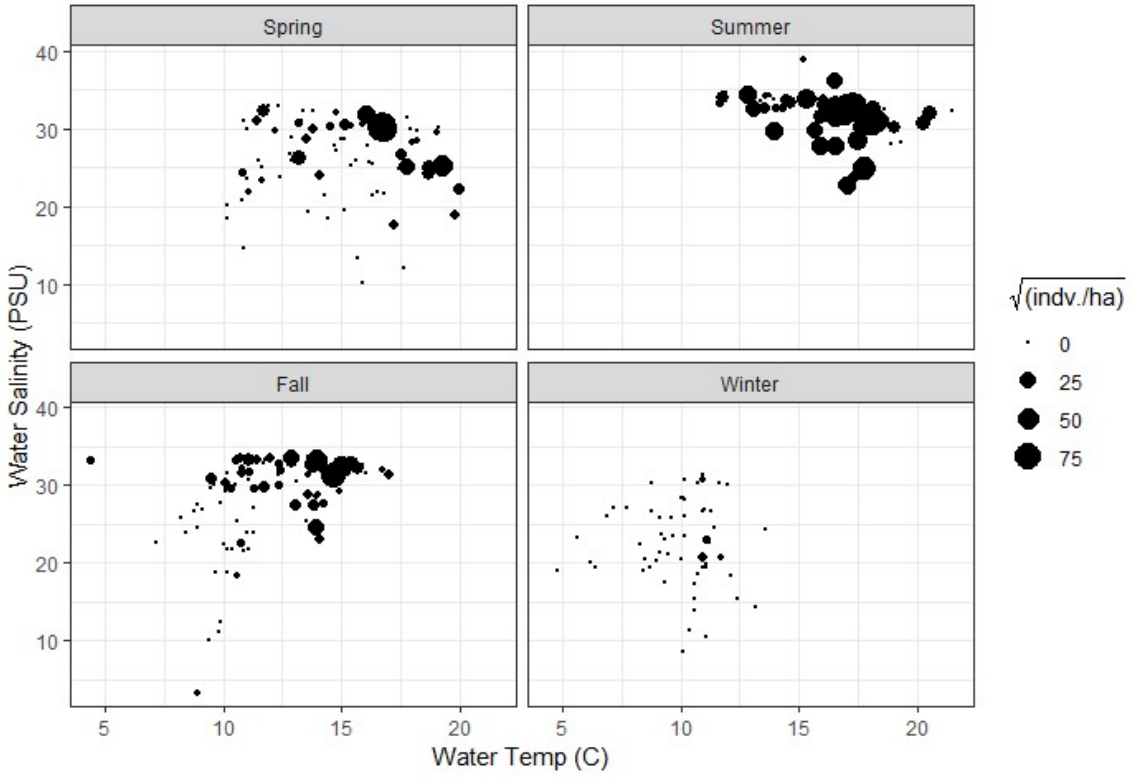

Spring
Summer
Fall
Winter
Water Salinity (PSU)
Water Temp (C)
40
30
20
10
5
10
15
20
√(indv./ha)
0
25
50
75

Figure 9

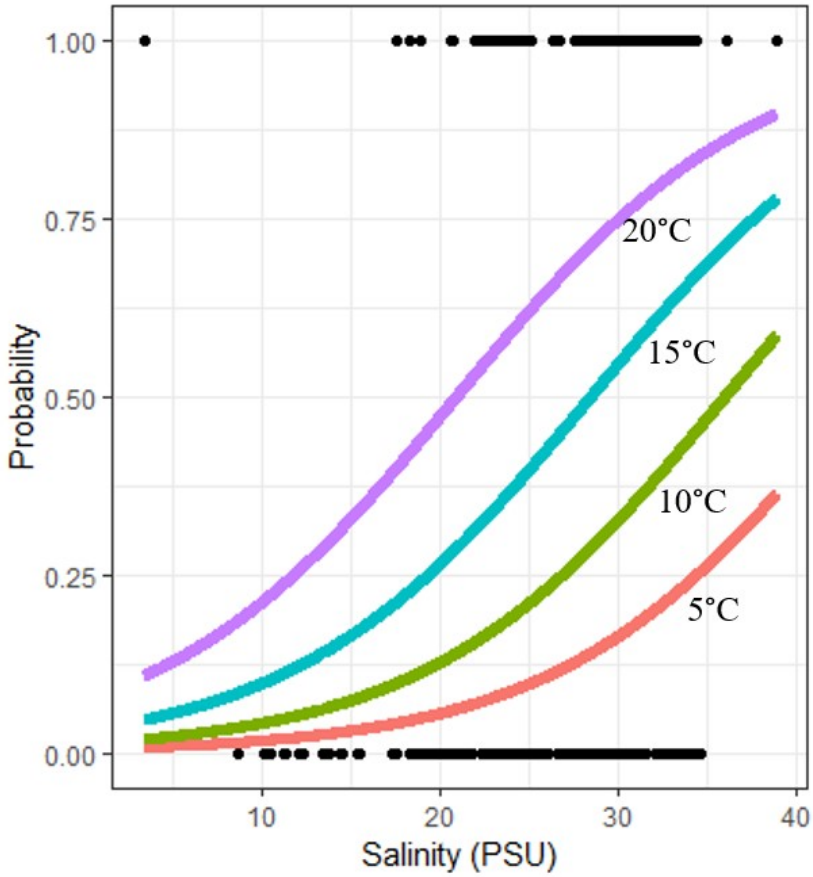

1.00
0.75
0.50
0.25
0.00
Probability
20°C
15°C
10°C
5°C
10
20
30
40
Salinity (PSU)

Figure 10

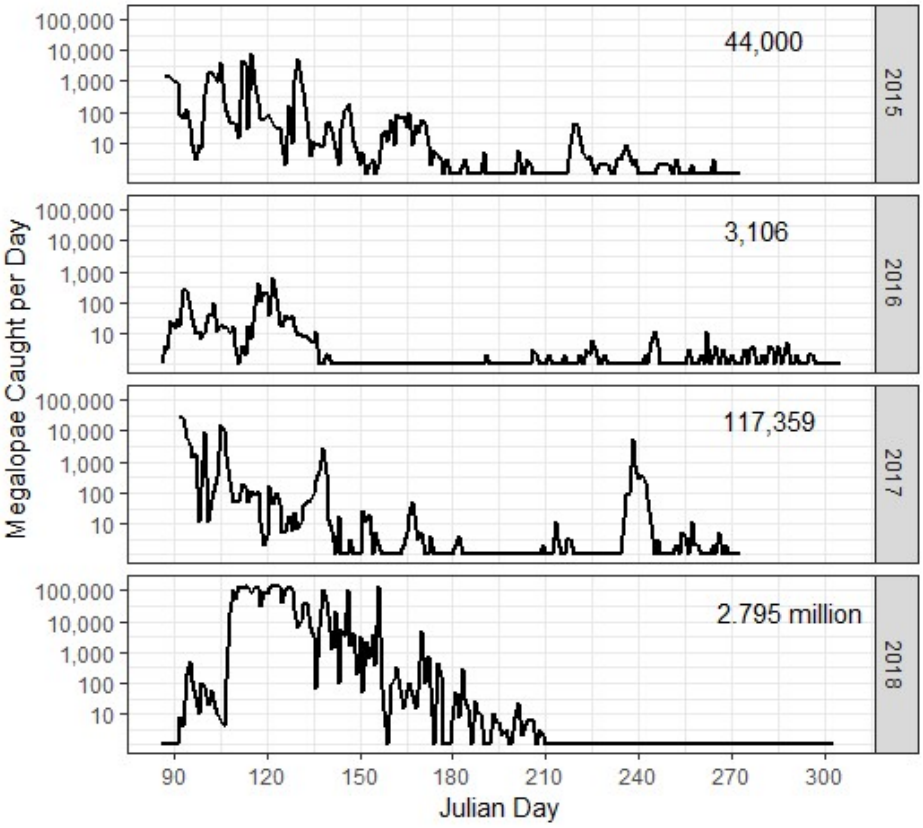

Megalopae Caught per Day
100,000
10,000
1,000
100
10
44,000
2015
3,106
2016
117,359
2017
2.795 million
2018
90
120
150
180
210
240
270
300
Julian Day

Figure 11

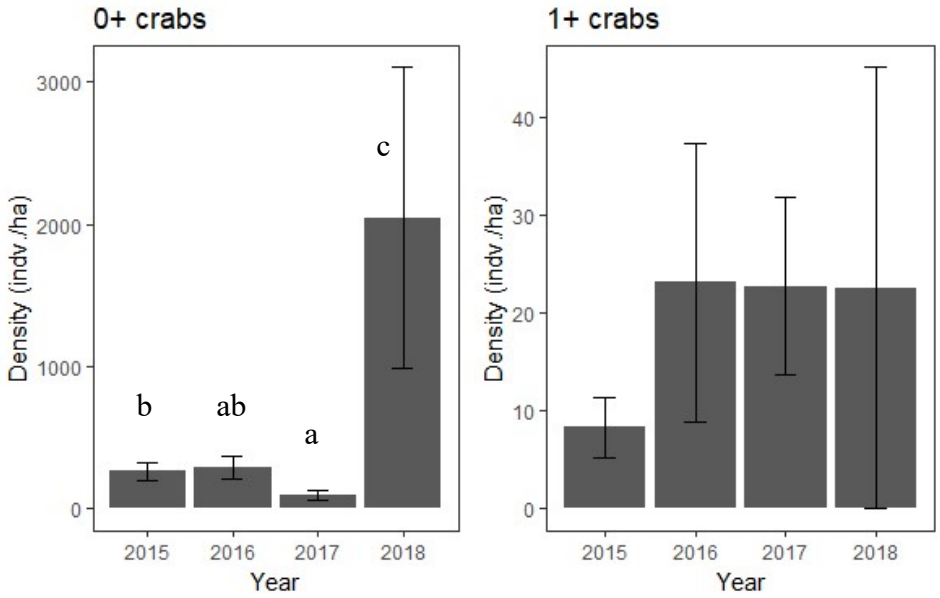

0+ crabs
1+ crabs
Density (indv./ha)
Year
2015
2016
2017
2018
3000
2000
1000
0
40
30
20
10
b
ab
a
c

Figure 12

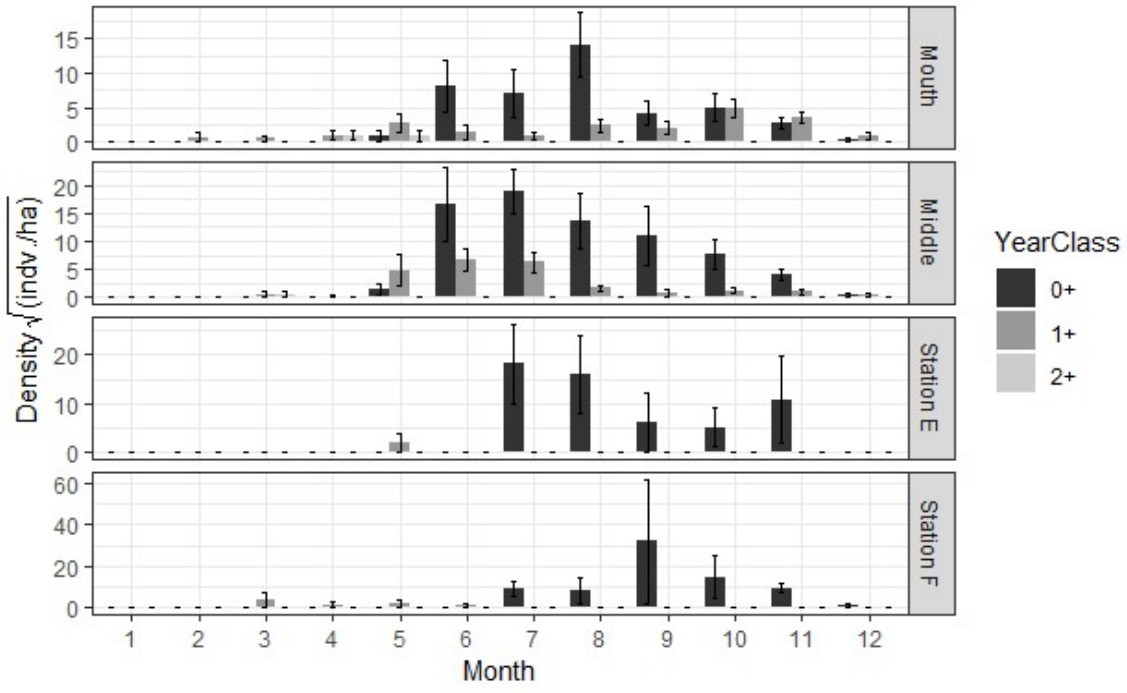

Mouth
Middle
Station E
Station F
Density $\sqrt{(indv./ha)}$
Month
YearClass
0+
1+
2+

Figure 13

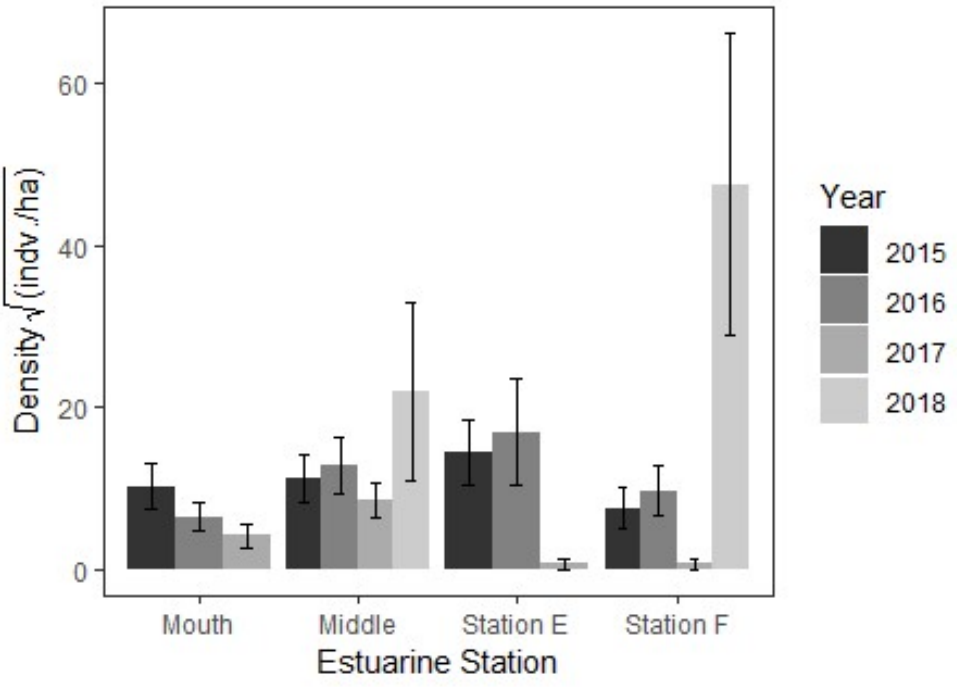

Density √(indv./ha)
60
40
20
0
Mouth
Middle
Station E
Station F
Estuarine Station
Year
2015
2016
2017
2018

Figure 14

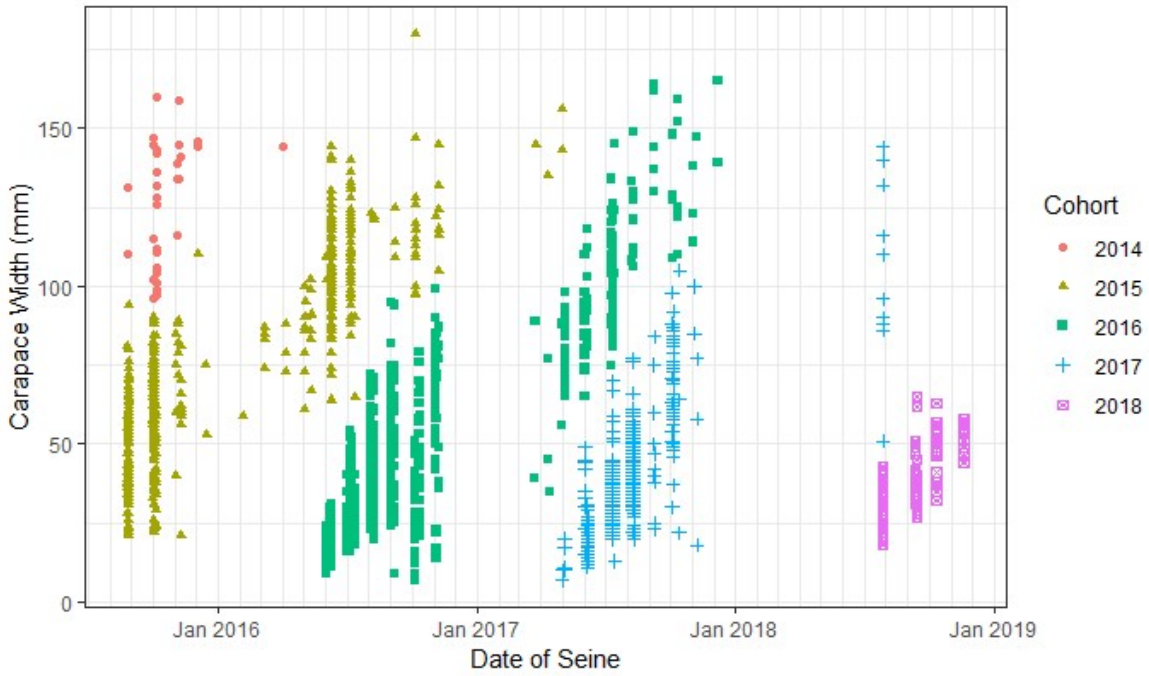

Carapace Width (mm)
150
100
50
0
Jan 2016
Jan 2017
Jan 2018
Jan 2019
Date of Seine
Cohort
2014
2015
2016
2017
2018

Figure 15

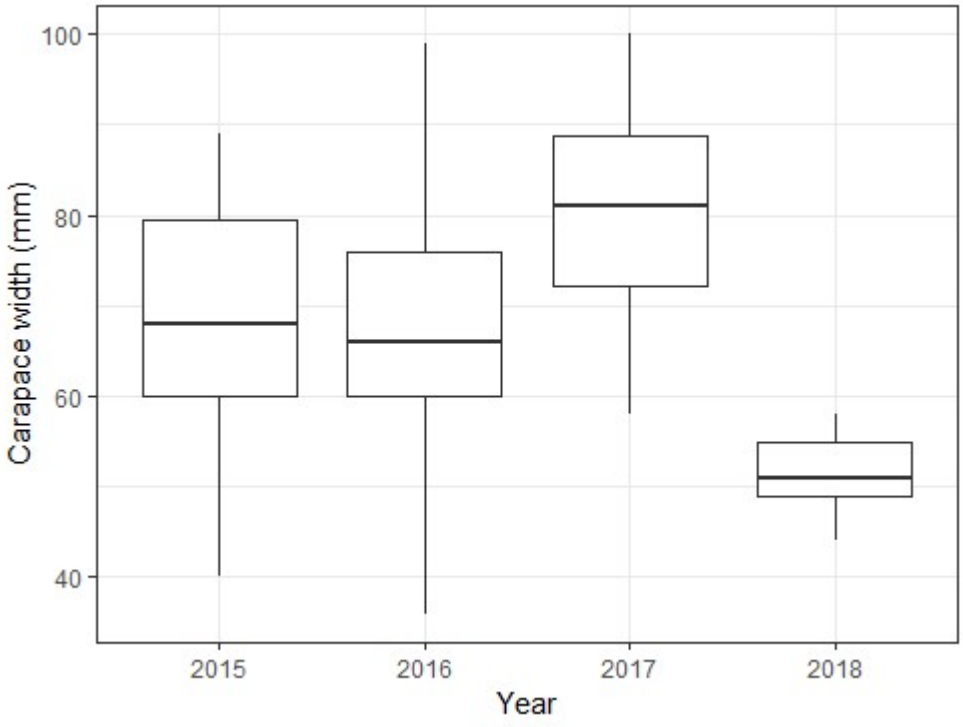


Figure 16

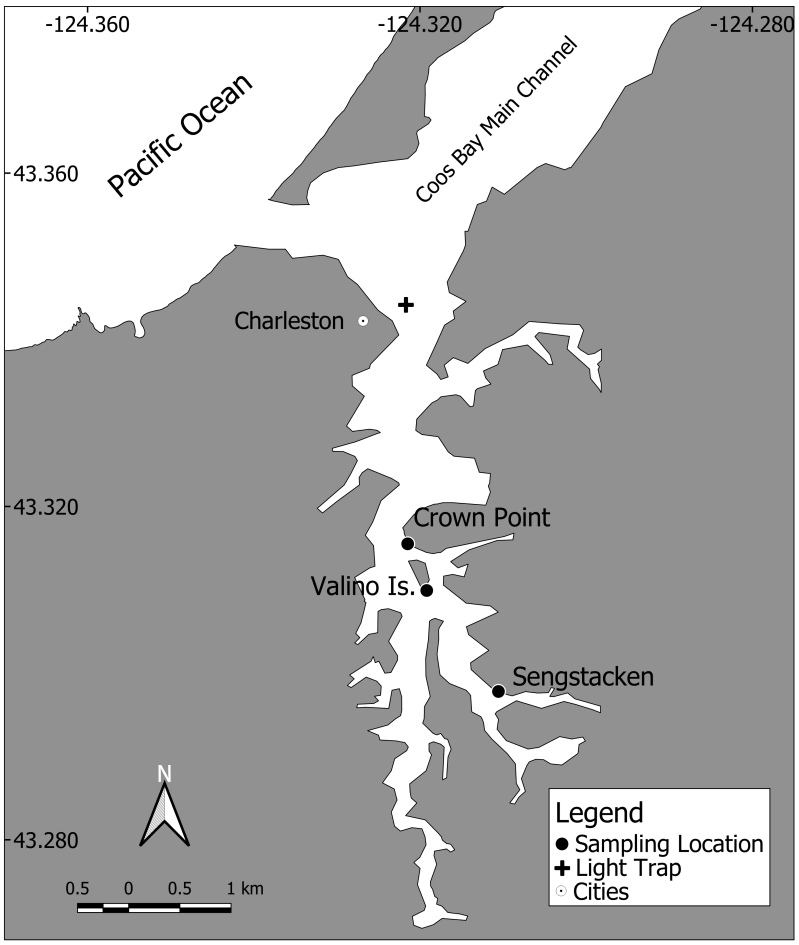

-124.360
-124.320
-124.280
43.360
43.320
43.280
Pacific Ocean
Coos Bay Main Channel
Charleston
Crown Point
Valino Is.
Sengstacken
N
0.5 0 0.5 1 km
Legend
Sampling Location
Light Trap
Cities

Figure 17

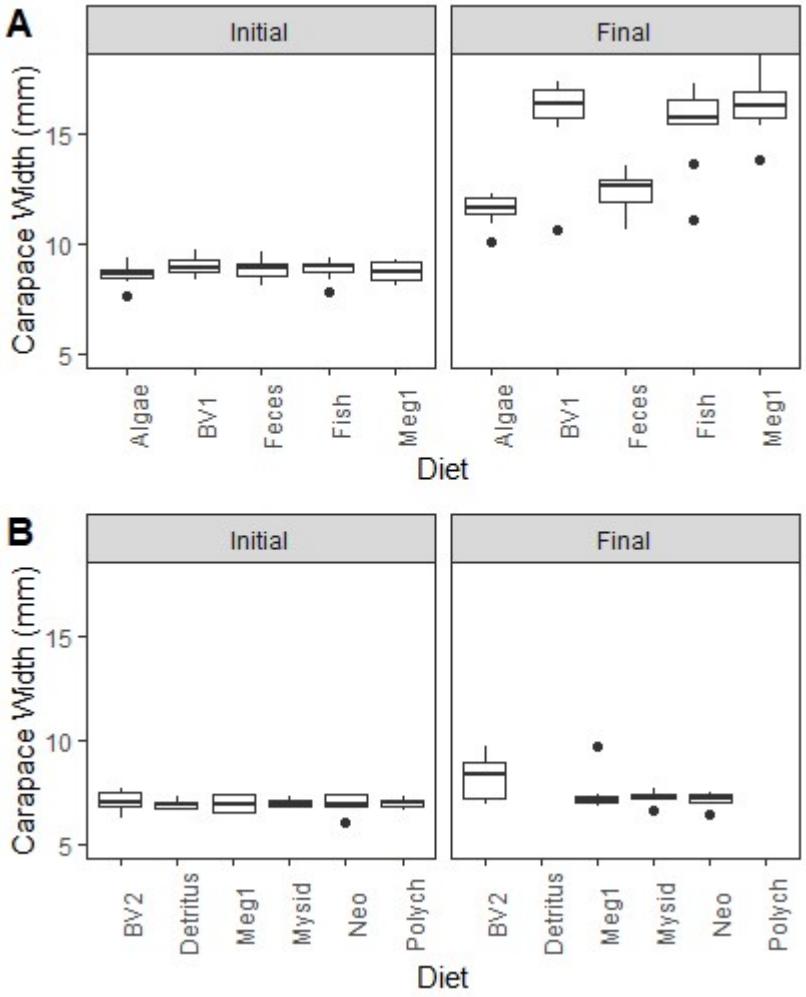

A
Initial
Final
Carapace Width (mm)
15
10
5
Algae
BV1
Feces
Fish
Meg1
Diet
B
Initial
Final
Carapace Width (mm)
15
10
5
BV2
Detritus
Meg1
Mysid
Neo
Polych
Diet

Figure 18

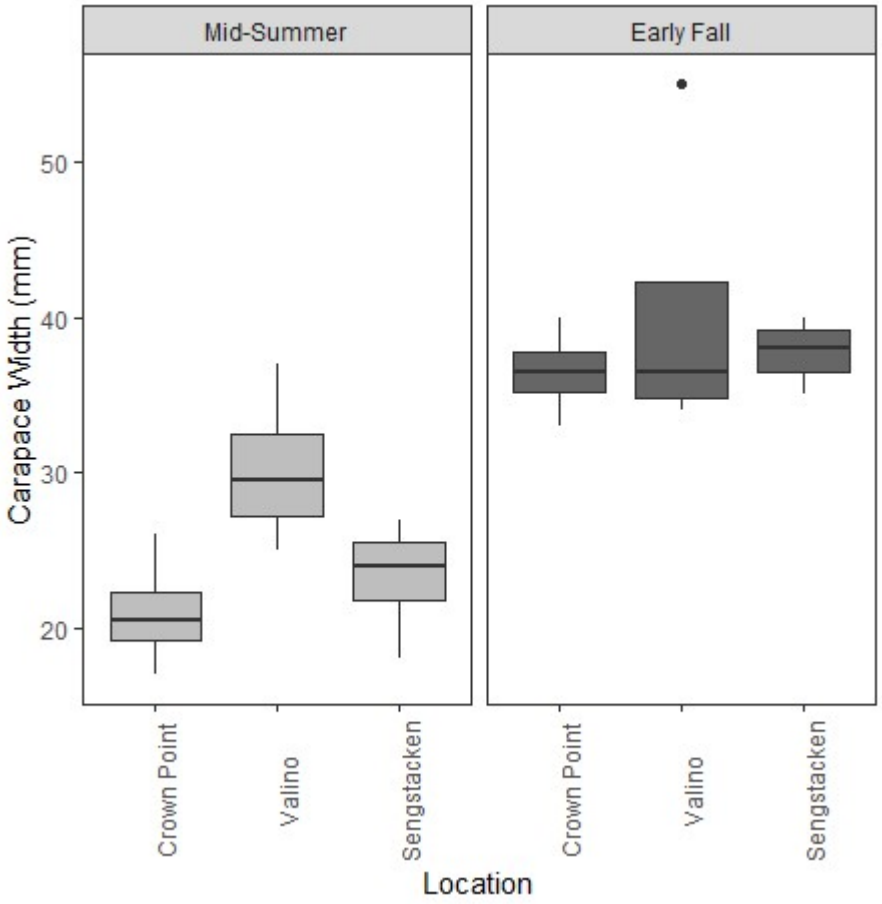

Mid-Summer
Early Fall
Carapace Width (mm)
50
40
30
20
Crown Point
Valino
Sengstacken
Crown Point
Valino
Sengstacken
Location

Figure 19

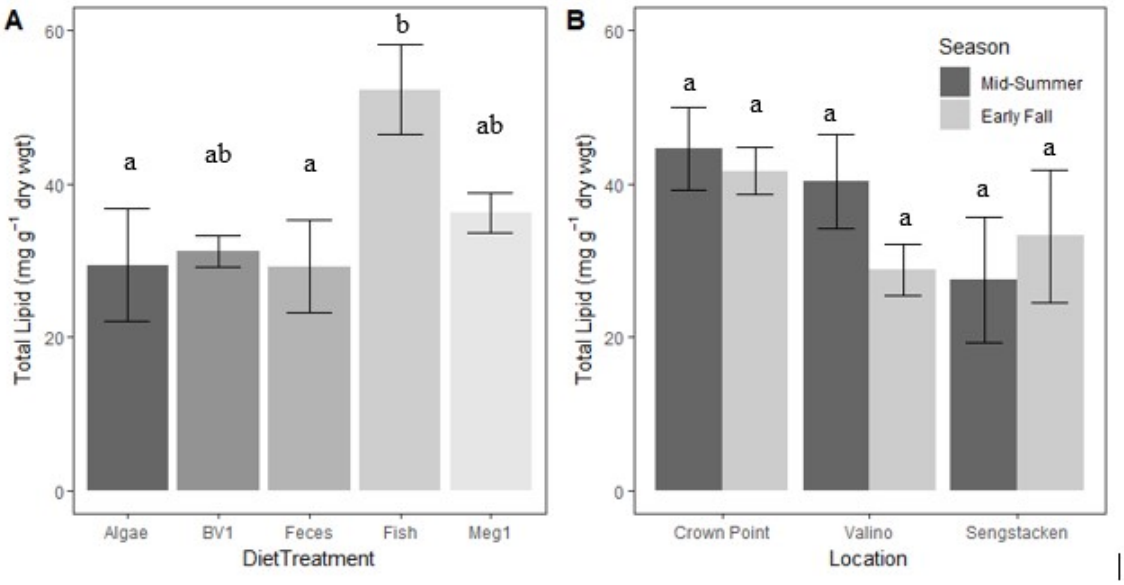

A
B
Total Lipid (mg g$^{-1}$ dry wgt)
60
40
20
0
a
ab
a
b
ab
Algae
BV1
Feces
Fish
Meg1
DietTreatment
Season
Mid-Summer
Early Fall
a
a
a
a
a
a
Crown Point
Valino
Sengstacken
Location

Figure 20

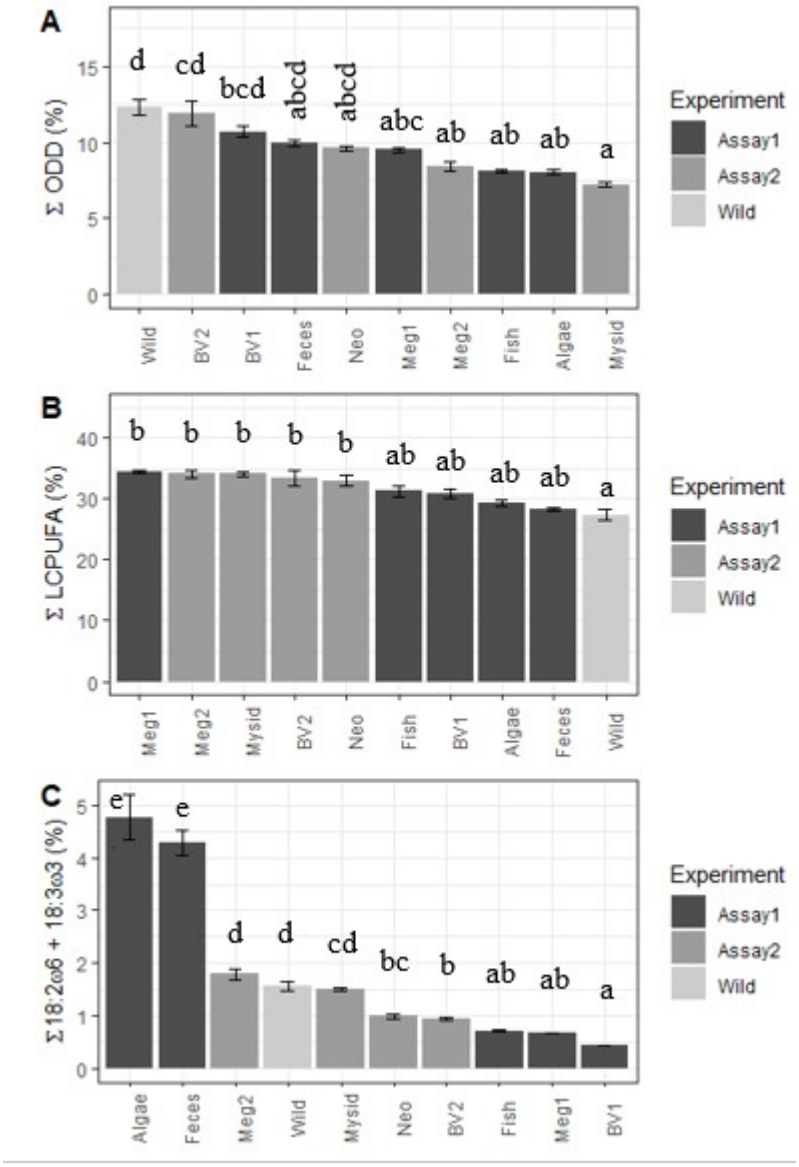

A
Σ ODD (%)
d cd bcd abcd abcd abc ab ab ab a
Wild BV2 BV1 Feces Neo Meg1 Meg2 Fish Algae Mysid
B
Σ LCPUFA (%)
b b b b b ab ab ab ab a
Meg1 Meg2 Mysid BV2 Neo Fish BV1 Algae Feces Wild
C
Σ 18:2ω6 + 18:3ω3 (%)
e e d d cd bc b ab ab a
Algae Feces Meg2 Wild Mysid Neo BV2 Fish Meg1 BV1
Experiment
Assay1
Assay2
Wild

Figure 21

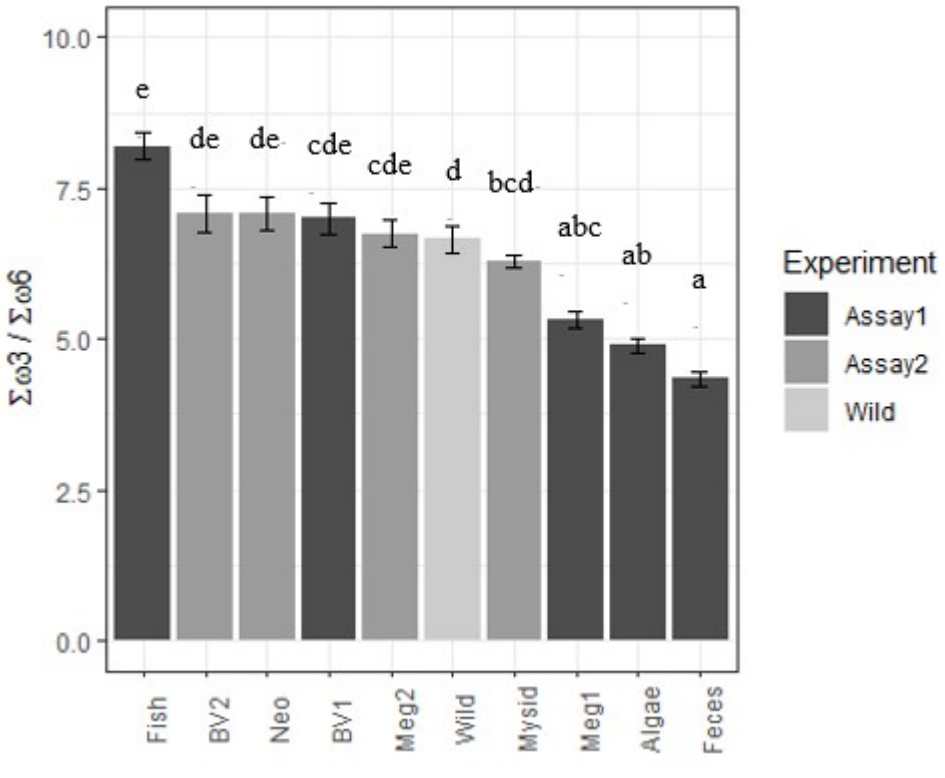

10.0
7.5
5.0
2.5
0.0
Σω3 / Σω6
e
de
de
cde
cde
d
bcd
abc
ab
a
Fish
BV2
Neo
BV1
Meg2
Wild
Mysid
Meg1
Algae
Feces
Experiment
Assay1
Assay2
Wild

Figure 22

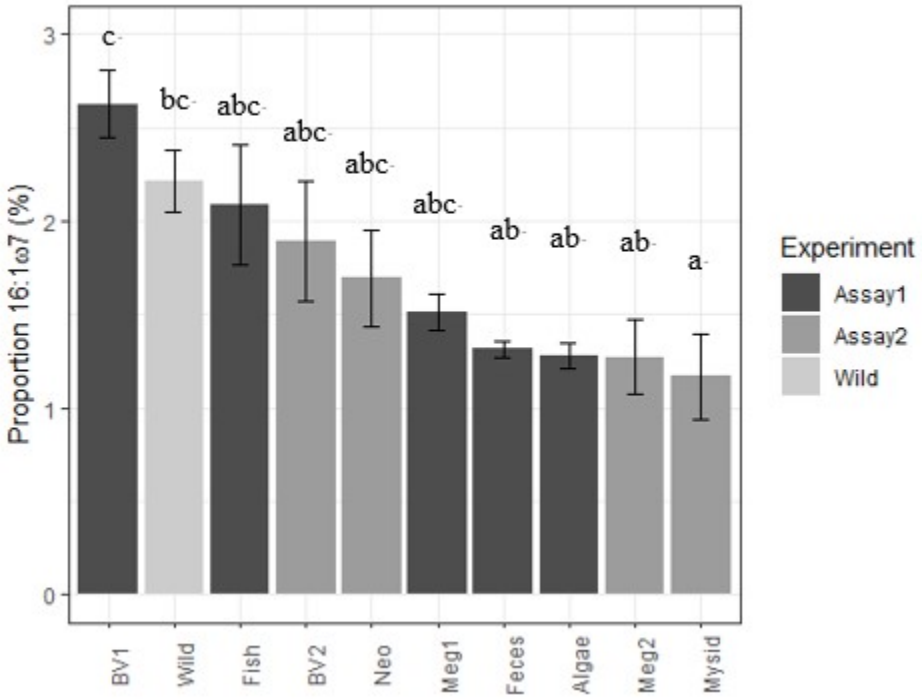

Proportion 16:1ω7 (%)
3
2
1
0
c
bc
abc
abc
abc
abc
ab
ab
ab
a
BV1
Wild
Fish
BV2
Neo
Meg1
Feces
Algae
Meg2
Mysid
Experiment
Assay1
Assay2
Wild

Figure 23

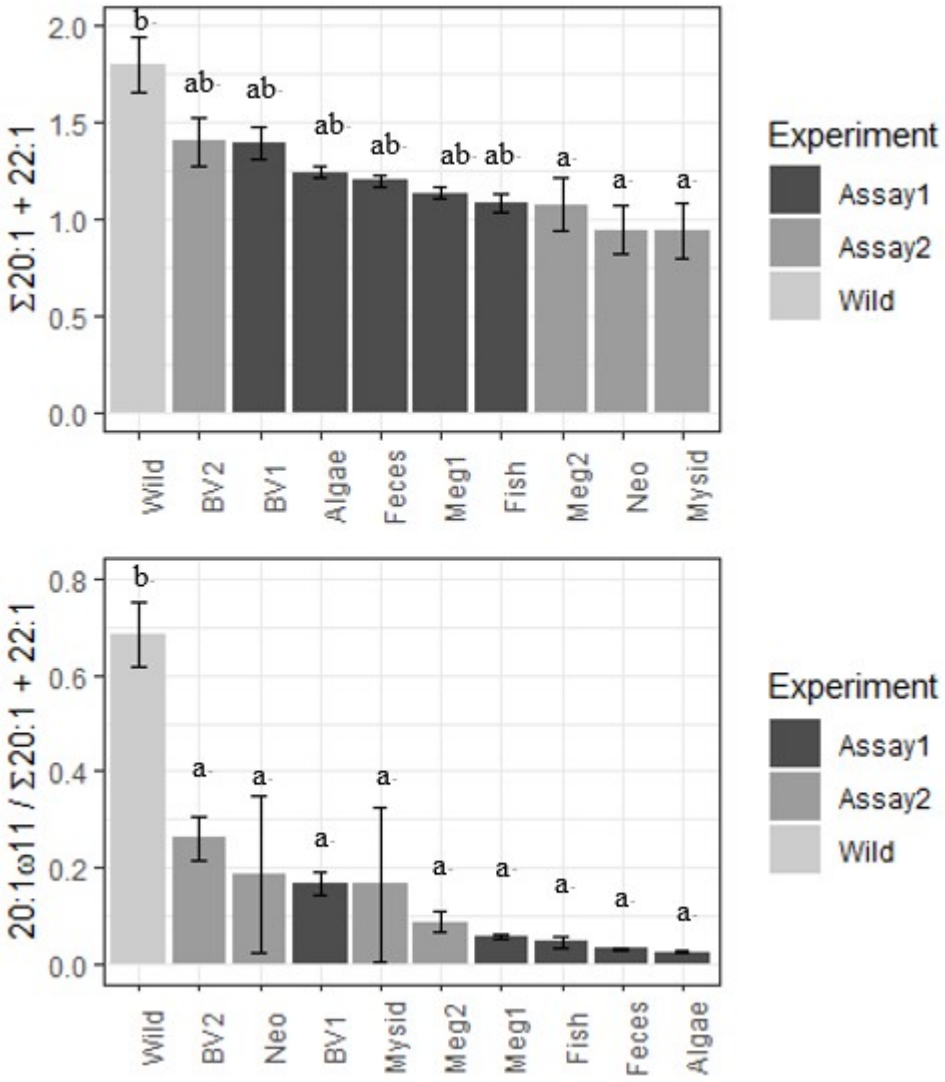

Σ20:1 + 22:1
2.0
1.5
1.0
0.5
0.0
b
ab
ab
ab
ab
ab
ab
a
a
a
Wild
BV2
BV1
Algae
Feces
Meg1
Fish
Meg2
Neo
Mysid
Experiment
Assay1
Assay2
Wild
20:1ω11 / Σ20:1 + 22:1
0.8
0.6
0.4
0.2
0.0
b
a
a
a
a
a
a
a
a
a
Wild
BV2
Neo
BV1
Mysid
Meg2
Meg1
Fish
Feces
Algae
Experiment
Assay1
Assay2
Wild

Figure 24

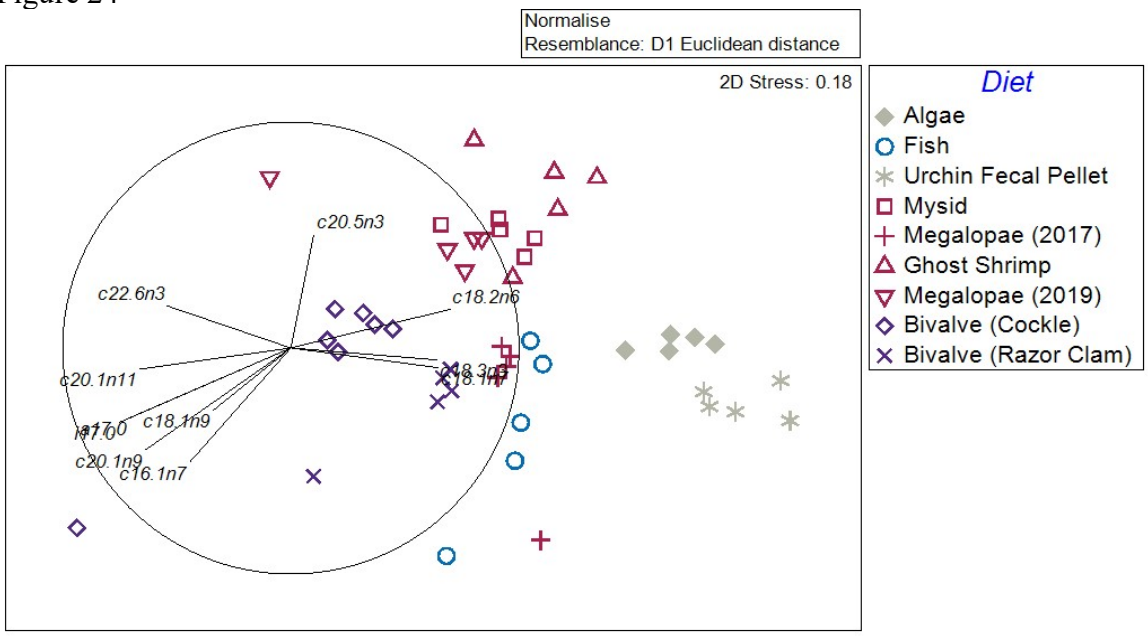

Normalise
Resemblance: D1 Euclidean distance
2D Stress: 0.18
c20.5n3
c22.6n3
c18.2n6
c20.1n11
c18.1n9
c16.1n7
Diet
Algae
Fish
Urchin Fecal Pellet
Mysid
Megalopae (2017)
Ghost Shrimp
Megalopae (2019)
Bivalve (Cockle)
Bivalve (Razor Clam)

Figure 25

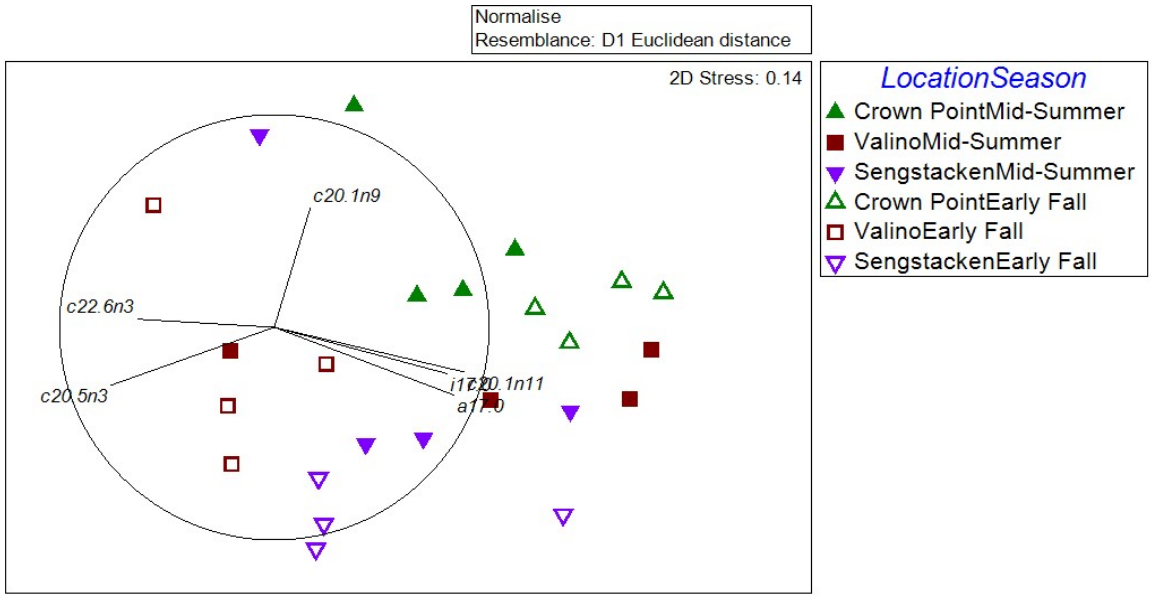
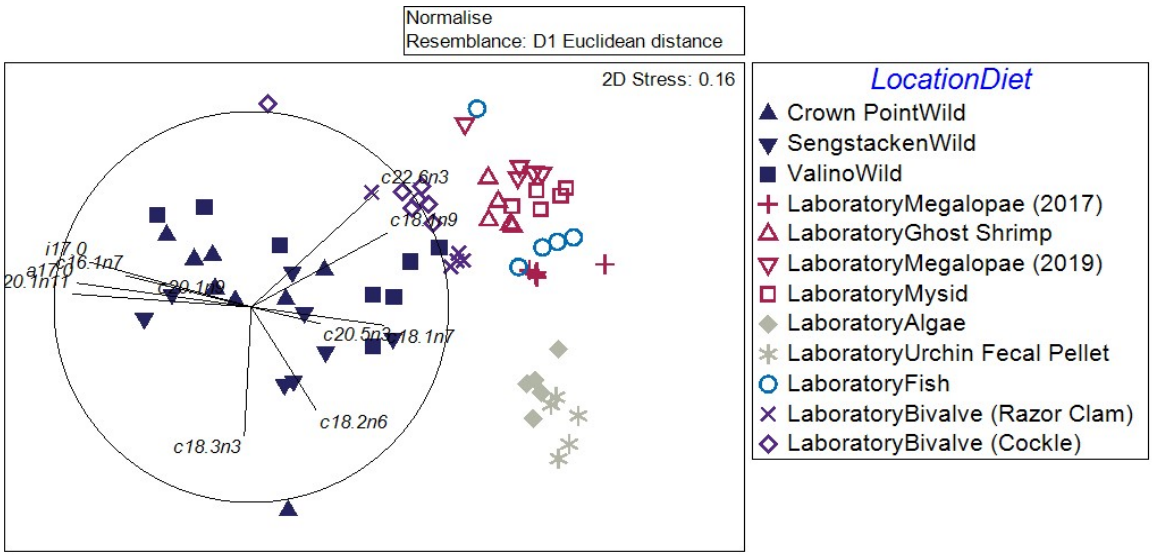

Normalise
Resemblance: D1 Euclidean distance
2D Stress: 0.14
c20.1n9
c22.6n3
c20.5n3
LocationSeason
Crown PointMid-Summer
ValinoMid-Summer
SengstackenMid-Summer
Crown PointEarly Fall
ValinoEarly Fall
SengstackenEarly Fall

Figure 26


Normalise
Resemblance: D1 Euclidean distance
2D Stress: 0.16
c22.6n3
c18.4n9
i17.0
a17.0
c16.1n7
20.1n11
c20.1n9
c20.5n3
18.1n7
c18.2n6
c18.3n3
LocationDiet
Crown PointWild
SengstackenWild
ValinoWild
LaboratoryMegalopae (2017)
LaboratoryGhost Shrimp
LaboratoryMegalopae (2019)
LaboratoryMysid
LaboratoryAlgae
LaboratoryUrchin Fecal Pellet
LaboratoryFish
LaboratoryBivalve (Razor Clam)
LaboratoryBivalve (Cockle)

APPENDIX C: TABLES

**Table 1 –** Results of one-way PERMANOVA (Euclidean distance) of FA proportion (24 FA contributing > 1% to all FA identified) from juvenile Dungeness crabs fed mono-specific diets in both feeding assays.

| Variable | df | MS | Pseudo-F | P (perm) | Unique Permutations |
|---|---|---|---|---|---|
| Diet | 8 | 109.87 | 18.556 | 0.0001 | 9865 |
| Residual | 38 | 5.9211 | | | |

**Table 2 –** Results of one-way PERMANOVA (Euclidean distance) of FA proportion (24 FA contributing > 1% to all FA identified) from wild juvenile Dungeness crabs caught at three locations in the South Slough estuary in July 2018 and September 2018.

| Variable | df | MS | Pseudo-F | P (perm) | Unique Permutations |
|---|---|---|---|---|---|
| Location | 2 | 71.761 | 4.4501 | 0.0003 | 9929 |
| Season | 1 | 19.983 | 1.2392 | 0.2644 | 9936 |
| Location x Season | 2 | 49.117 | 3.0459 | 0.0028 | 9925 |
| Residual | 18 | 16.126 | | | |

**Table 3 –** Results of one-way PERMANOVA (Euclidean distance) of FA proportion (24 FA contributing > 1% to all FA identified) from both wild-caught and experimental juvenile Dungeness crabs fed mono-specific diets.

| Variable | df | MS | Pseudo-F | P (perm) | Unique Permutations |
|---|---|---|---|---|---|
| Diet | 9 | 129.12 | 15.207 | 0.0001 | 9881 |
| Residual | 61 | 8.4906 | | | |

**Table 4 –** Fatty acid composition of juvenile Dungeness crabs in Feeding Assay 1. Diet codes are: Algae = *Ulva sp.*, Feces = *Strongylocentrotus purpuratus* feces, BV1 = *Siliqua patula*, Meg1 = *M. magister megalopae*, Fish = *Sebastes melanops*. Fatty acids shown are those that contribute > 1% to all FA identified. Numbers are proportion of all FA identified ± SE.

**Feeding Assay 1**

| | Algae | Feces | BV1 | Meg1 | Fish |
|---|---|---|---|---|---|
| c14.0 | 0.4 ± 0.06 | 0.3 ± 0.02 | 0.5 ± 0.06 | 0.2 ± 0.02 | 0.5 ± 0.16 |
| c16.0 | 27.4 ± 0.29 | 30.1 ± 0.36 | 25.7 ± 0.28 | 25 ± 0.19 | 29.2 ± 0.29 |
| c18.0 | 12.9 ± 0.14 | 10.9 ± 0.26 | 12.8 ± 0.19 | 13.1 ± 0.13 | 12.1 ± 0.33 |
| c20.0 | 1.3 ± 0.06 | 1.3 ± 0.04 | 0.9 ± 0.04 | 0.8 ± 0.02 | 0.8 ± 0.05 |
| c22.0 | 2.1 ± 0.11 | 2.2 ± 0.07 | 1 ± 0.06 | 1 ± 0.03 | 0.9 ± 0.09 |
| c24.0 | 0.2 ± 0.01 | 0.2 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.005 | 0.1 ± 0.01 |
| **Σ SAFA** | **44.4 ± 0.34** | **43.7 ± 0.6** | **41 ± 0.25** | **44.9 ± 0.41** | **43.7 ± 0.4** |
| c16.1n7 | 1.3 ± 0.07 | 1.3 ± 0.05 | 2.6 ± 0.18 | 1.5 ± 0.1 | 2.1 ± 0.32 |
| c18.1n9 | 7.2 ± 0.36 | 6 ± 0.09 | 8.5 ± 0.17 | 9.1 ± 0.13 | 9.6 ± 0.31 |
| c18.1n7 | 5.8 ± 0.16 | 7.1 ± 0.13 | 5.5 ± 0.06 | 6 ± 0.15 | 5.4 ± 0.14 |
| c20.1n11 | 0.03 ± 0.002 | 0.04 ± 0.002 | 0.2 ± 0.04 | 0.1 ± 0.01 | 0.05 ± 0.01 |
| c20.1n9 | 0.8 ± 0.02 | 0.8 ± 0.02 | 0.8 ± 0.04 | 0.9 ± 0.02 | 0.8 ± 0.05 |
| c20.1n7 | 0.2 ± 0.004 | 0.2 ± 0.01 | 1.2 ± 0.18 | 0.4 ± 0.03 | 0.3 ± 0.02 |
| **Σ MUFA** | **10.1 ± 0.41** | **11.1 ± 0.24** | **14.1 ± 0.46** | **8.9 ± 0.12** | **13.6 ± 0.73** |
| c18.2n6 | 1.9 ± 0.14 | 2.4 ± 0.09 | 0.3 ± 0.005 | 0.6 ± 0.01 | 0.6 ± 0.01 |
| c18.3n3 | 2.9 ± 0.29 | 1.9 ± 0.14 | 0.1 ± 0.003 | 0.1 ± 0.003 | 0.1 ± 0.01 |
| c18.4n1 | 0.2 ± 0.02 | 0.1 ± 0.01 | 0.03 ± 0.003 | 0.02 ± 0.001 | 0.04 ± 0.01 |
| c20.2n6 | 1.2 ± 0.04 | 1.1 ± 0.04 | 1.2 ± 0.04 | 1.4 ± 0.02 | 1 ± 0.08 |
| c20.4n6 | 2.6 ± 0.07 | 2.7 ± 0.06 | 2.4 ± 0.08 | 3.8 ± 0.07 | 2 ± 0.1 |
| c20.5n3 | 16.6 ± 0.38 | 15.8 ± 0.13 | 15.1 ± 0.57 | 19.5 ± 0.27 | 14.1 ± 0.73 |
| c22.5n3 | 1 ± 0.14 | 1.5 ± 0.1 | 0.7 ± 0.04 | 0.6 ± 0.02 | 0.9 ± 0.1 |
| c22.6n3 | 10.1 ± 0.09 | 9.8 ± 0.29 | 13.5 ± 0.22 | 11.2 ± 0.13 | 15.2 ± 0.28 |
| **Σ PUFA** | **37.5 ± 0.44** | **35.6 ± 0.77** | **34.1 ± 0.7** | **36.2 ± 0.38** | **34.6 ± 0.81** |
| c15.0 | 0.5 ± 0.03 | 1.1 ± 0.08 | 0.6 ± 0.03 | 0.4 ± 0.02 | 0.4 ± 0.02 |
| i17.0 | 0.3 ± 0.01 | 0.3 ± 0.01 | 0.9 ± 0.08 | 0.7 ± 0.05 | 0.6 ± 0.04 |
| a17.0 | 0.1 ± 0.004 | 0.1 ± 0.002 | 0.3 ± 0.02 | 0.2 ± 0.02 | 0.1 ± 0.01 |
| c17.0 | 0.7 ± 0.01 | 0.7 ± 0.03 | 1.9 ± 0.11 | 0.9 ± 0.22 | 0.6 ± 0.05 |
| **Σ Odd** | **8.1 ± 0.17** | **9.6 ± 0.15** | **10.7 ± 0.38** | **10 ± 0.2** | **8.1 ± 0.15** |
| **Σ EFA** | **4.8 ± 0.43** | **1 ± 0.05** | **0.4 ± 0.01** | **4.3 ± 0.23** | **0.7 ± 0.02** |
| **Σ LCPUFA** | **29.4 ± 0.5** | **33 ± 0.84** | **30.9 ± 0.78** | **28.4 ± 0.3** | **31.3 ± 0.82** |

**Table 5 –** Fatty acid composition of juvenile Dungeness crabs in Feeding Assay 2. . Diet codes are: BV2 = *Clinocardium nuttalii*, Meg2 = *M. magister* megalopae, Mysid = *Neomysis mercedes*, Neo = *Neotrypaea californiensis*,. Fatty acids shown are those that contribute > 1% to all FA identified. Numbers are proportion of all FA identified ± SE.

**Feeding Assay 2**

| | BV2 | Meg2 | Mysid | Neo |
|---|---|---|---|---|
| c14.0 | 0.4 ± 0.06 | 0.7 ± 0.19 | 0.5 ± 0.17 | 0.7 ± 0.17 |
| c16.0 | 24.5 ± 0.25 | 26.7 ± 0.36 | 28.6 ± 0.31 | 25.6 ± 0.49 |
| c18.0 | 12.3 ± 0.38 | 12.5 ± 0.38 | 12.7 ± 0.35 | 15 ± 0.16 |
| c20.0 | 0.4 ± 0.02 | 0.4 ± 0.01 | 0.5 ± 0.01 | 0.5 ± 0.02 |
| c22.0 | 0.5 ± 0.04 | 0.5 ± 0.02 | 0.6 ± 0.02 | 0.6 ± 0.03 |
| c24.0 | 1.5 ± 0.05 | 1.8 ± 0.02 | 1.6 ± 0.03 | 1.3 ± 0.06 |
| **Σ SAFA** | **39.5 ± 0.63** | **40.2 ± 0.14** | **42.5 ± 0.57** | **44.6 ± 0.38** |
| c16.1n7 | 1.9 ± 0.32 | 1.3 ± 0.2 | 1.2 ± 0.23 | 1.7 ± 0.26 |
| c18.1n9 | 7.2 ± 0.23 | 7.1 ± 0.15 | 8 ± 0.09 | 7.8 ± 0.1 |
| c18.1n7 | 4.6 ± 0.07 | 5.5 ± 0.14 | 4.7 ± 0.15 | 6.8 ± 0.06 |
| c20.1n11 | 0.3 ± 0.07 | 0.1 ± 0.03 | 0.1 ± 0.13 | 0.1 ± 0.13 |
| c20.1n9 | 1 ± 0.05 | 0.9 ± 0.08 | 0.7 ± 0.01 | 0.7 ± 0.01 |
| c20.1n7 | 0.7 ± 0.17 | 0.2 ± 0.04 | 0.1 ± 0.01 | 0.2 ± 0.01 |
| **Σ MUFA** | **11.6 ± 0.87** | **12.6 ± 0.24** | **10.2 ± 0.47** | **10.5 ± 0.34** |
| c18.2n6 | 0.6 ± 0.02 | 1.3 ± 0.06 | 1.3 ± 0.02 | 0.8 ± 0.04 |
| c18.3n3 | 0.3 ± 0.02 | 0.4 ± 0.04 | 0.3 ± 0.02 | 0.2 ± 0.01 |
| c18.4n1 | 0.05 ± 0.01 | 0.1 ± 0.03 | 0.04 ± 0.02 | 0.1 ± 0.02 |
| c20.2n6 | 1.1 ± 0.03 | 1.5 ± 0.06 | 1.3 ± 0.05 | 0.8 ± 0.04 |
| c20.4n6 | 2.6 ± 0.09 | 2 ± 0.04 | 2.6 ± 0.1 | 2.8 ± 0.15 |
| c20.5n3 | 17.6 ± 0.86 | 15.9 ± 0.56 | 17.2 ± 0.12 | 19.4 ± 0.33 |
| c22.5n3 | 0.7 ± 0.12 | 0.2 ± 0.04 | 0.2 ± 0.01 | 0.2 ± 0.02 |
| c22.6n3 | 13.2 ± 0.45 | 16.3 ± 0.25 | 14.3 ± 0.29 | 10.9 ± 0.56 |
| **Σ PUFA** | **37 ± 1.12** | **37.6 ± 0.26** | **38.8 ± 0.42** | **37.7 ± 0.37** |
| c15.0 | 1 ± 0.12 | 0.7 ± 0.07 | 0.4 ± 0.02 | 0.4 ± 0.02 |
| i17.0 | 2.5 ± 0.33 | 0.3 ± 0.05 | 0.3 ± 0.02 | 0.4 ± 0.03 |
| a17.0 | 0.9 ± 0.14 | 0.1 ± 0.01 | 0.1 ± 0.01 | 0.1 ± 0.01 |
| c17.0 | 1.4 ± 0.03 | 1.3 ± 0.03 | 1.4 ± 0.03 | 1.1 ± 0.04 |
| **Σ Odd** | **11.9 ± 0.84** | **9.5 ± 0.13** | **8.4 ± 0.33** | **7.2 ± 0.14** |
| **Σ EFA** | **0.9 ± 0.02** | **0.7 ± 0.01** | **1.8 ± 0.09** | **1.5 ± 0.03** |
| **Σ LCPUFA** | **33.4 ± 1.32** | **34.4 ± 0.29** | **34.1 ± 0.66** | **34.1 ± 0.4** |

**Table 6 –** Fatty acid composition of wild juvenile Dungeness crabs caught in the South Slough estuary. Fatty acids shown are those that contribute > 1% to all FA identified. Numbers are proportion of all FA identified ± SE.

| | Crown Point | | Valino | | Sengstacken | |
|---|---|---|---|---|---|---|
| | Mid-Summer | Early Fall | Mid-Summer | Early Fall | Mid-Summer | Early Fall |
| c14.0 | 2.4 ± 0.27 | 2.2 ± 0.17 | 1.8 ± 0.5 | 0.6 ± 0.19 | 1.7 ± 0.5 | 1.2 ± 0.53 |
| c16.0 | 26.7 ± 0.54 | 26.5 ± 0.65 | 25.2 ± 0.19 | 25.1 ± 1.32 | 26.1 ± 0.96 | 23.3 ± 0.42 |
| c18.0 | 15 ± 0.96 | 13.6 ± 0.92 | 12.7 ± 0.2 | 14.2 ± 0.9 | 14.3 ± 0.89 | 16 ± 0.9 |
| c20.0 | 0.8 ± 0.04 | 0.6 ± 0.06 | 0.7 ± 0.03 | 0.7 ± 0.05 | 0.8 ± 0.06 | 0.9 ± 0.03 |
| c22.0 | 0.9 ± 0.06 | 0.6 ± 0.04 | 0.8 ± 0.06 | 0.9 ± 0.05 | 0.9 ± 0.1 | 1.1 ± 0.07 |
| c24.0 | 0.2 ± 0.03 | 0.1 ± 0.01 | 1.3 ± 0.11 | 1.4 ± 0.08 | 1.2 ± 0.06 | 1.1 ± 0.05 |
| **Σ SAFA** | **46.1 ± 0.26** | **43.7 ± 0.34** | **42.3 ± 0.32** | **42.9 ± 1.03** | **45 ± 0.58** | **43.6 ± 0.53** |
| c16.1n7 | 2.5 ± 0.28 | 3.2 ± 0.28 | 2.1 ± 0.32 | 1.2 ± 0.13 | 2.4 ± 0.43 | 1.9 ± 0.36 |
| c18.1n9 | 5.5 ± 0.66 | 5 ± 0.22 | 5.8 ± 0.26 | 6.6 ± 0.8 | 5.3 ± 0.51 | 4.7 ± 0.13 |
| c18.1n7 | 5 ± 0.42 | 4.4 ± 0.16 | 4 ± 0.1 | 4.3 ± 0.32 | 3.7 ± 0.17 | 3.8 ± 0.08 |
| c20.1n11 | 0.6 ± 0.12 | 1.1 ± 0.09 | 0.9 ± 0.21 | 0.3 ± 0.03 | 0.6 ± 0.17 | 0.7 ± 0.21 |
| c20.1n9 | 1.2 ± 0.56 | 1 ± 0.21 | 1 ± 0.1 | 0.7 ± 0.1 | 0.7 ± 0.08 | 0.6 ± 0.04 |
| c20.1n7 | 1.4 ± 0.08 | 2 ± 0.2 | 1.7 ± 0.3 | 0.7 ± 0.09 | 1.3 ± 0.24 | 1 ± 0.24 |
| **Σ MUFA** | **12.1 ± 0.77** | **13.1 ± 0.3** | **12.2 ± 0.65** | **10.1 ± 1.04** | **10.8 ± 0.91** | **9.5 ± 0.78** |
| c18.2n6 | 0.9 ± 0.17 | 0.8 ± 0.05 | 0.8 ± 0.02 | 1.1 ± 0.11 | 1 ± 0.24 | 0.8 ± 0.09 |
| c18.3n3 | 0.6 ± 0.15 | 0.9 ± 0.18 | 0.6 ± 0.14 | 0.6 ± 0.16 | 0.7 ± 0.16 | 0.5 ± 0.09 |
| c18.4n1 | 0.3 ± 0.05 | 0.6 ± 0.11 | 0.5 ± 0.19 | 0.1 ± 0.02 | 0.4 ± 0.15 | 0.2 ± 0.07 |
| c20.2n6 | 0.6 ± 0.05 | 0.5 ± 0.04 | 0.9 ± 0.06 | 0.7 ± 0.06 | 0.6 ± 0.06 | 0.6 ± 0.04 |
| c20.4n6 | 2 ± 0.21 | 1.7 ± 0.1 | 1.9 ± 0.1 | 2.7 ± 0.39 | 2.5 ± 0.16 | 3.2 ± 0.31 |
| c20.5n3 | 15.9 ± 0.74 | 14.8 ± 0.57 | 14.8 ± 1.91 | 18.8 ± 1.38 | 15.8 ± 0.98 | 17 ± 1.4 |
| c22.5n3 | 1.2 ± 0.12 | 1.4 ± 0.1 | 1.3 ± 0.06 | 1.1 ± 0.15 | 1.4 ± 0.24 | 1.7 ± 0.04 |
| c22.6n3 | 8.2 ± 0.56 | 7.7 ± 0.27 | 9.9 ± 1 | 11.1 ± 0.68 | 8.7 ± 0.58 | 7.6 ± 0.65 |
| **Σ PUFA** | **31.1 ± 0.74** | **29.7 ± 0.59** | **31.9 ± 2.56** | **37 ± 1.16** | **32.3 ± 1.1** | **32.6 ± 2.19** |
| c15.0 | 1.2 ± 0.25 | 1.5 ± 0.1 | 1.4 ± 0.23 | 0.8 ± 0.16 | 1.7 ± 0.39 | 2.1 ± 0.49 |
| i17.0 | 1.1 ± 0.12 | 2.5 ± 0.31 | 2.8 ± 0.66 | 1.1 ± 0.19 | 1.3 ± 0.24 | 1.4 ± 0.18 |
| a17.0 | 0.6 ± 0.08 | 1.2 ± 0.15 | 1.3 ± 0.37 | 0.5 ± 0.11 | 0.7 ± 0.17 | 0.8 ± 0.1 |
| c17.0 | 2.2 ± 0.06 | 2.5 ± 0.07 | 2.3 ± 0.18 | 2.3 ± 0.35 | 2.9 ± 0.36 | 4.2 ± 0.21 |
| **Σ Odd** | **10.8 ± 0.93** | **13.5 ± 0.61** | **13.6 ± 1.75** | **10 ± 0.55** | **11.9 ± 1.11** | **14.3 ± 1.13** |
| **Σ EFA** | **1.6 ± 0.32** | **1.6 ± 0.23** | **1.4 ± 0.15** | **1.6 ± 0.17** | **1.7 ± 0.4** | **1.4 ± 0.17** |
| **Σ LCPUFA** | **26.1 ± 0.97** | **24.3 ± 0.55** | **26.5 ± 2.98** | **32.7 ± 1.3** | **26.9 ± 1.6** | **27.7 ± 2.3** |

**Table 7 –** Fatty acid composition of food material fed to crabs in Feeding Assay 2. Diet codes are: BV2 = *Clinocardium nuttalii*, DT = detritus, MEG2 = *M. magister* megalopae, Mysid = *Neomysis mercedes*, Neo = *Neotrypaea californiensis*, PC = *Owenia sp.* Fatty acids shown are those that contribute > 1% to all FA identified. Numbers are proportion of all FA identified ± SE.

**Feeding Assay 2 Foods**

| | BV2 | DT | MEG2 | Mysid | Neo | PC |
|---|---|---|---|---|---|---|
| c14.0 | 2.2 ± 0.15 | 3.2 ± 0.45 | 9 ± 0.05 | 2 ± 0.15 | 3.4 ± 0 | 3.1 ± 0.3 |
| c16.0 | 29 ± 1.75 | 30.8 ± 0.55 | 25.4 ± 0.75 | 34.3 ± 0.2 | 27.4 ± 1.25 | 35.8 ± 1.15 |
| c18.0 | 13.9 ± 0.45 | 23.3 ± 0.6 | 8.1 ± 0.25 | 7 ± 0.55 | 10.3 ± 0.45 | 18.6 ± 1.85 |
| c20.0 | 0.2 ± 0.05 | 1.6 ± 0.05 | 0.5 ± 0.05 | 0.3 ± 0.05 | 0.8 ± 0.15 | 0.7 ± 0.1 |
| c22.0 | 0.05 ± 0.05 | 1.2 ± 0.05 | 0.3 ± 0 | 0.2 ± 0.05 | 0.6 ± 0.05 | 0.6 ± 0.05 |
| c24.0 | 1.4 ± 0 | 1.3 ± 0.1 | 1.3 ± 0 | 1.7 ± 0.05 | 0.9 ± 0.05 | 0.3 ± 0.1 |
| **Σ SAFA** | **46.6 ± 2.15** | **61.2 ± 0.5** | **44.6 ± 0.5** | **45.3 ± 0.55** | **43.1 ± 0.55** | **59 ± 2.65** |
| c16.1n7 | 1.9 ± 0.1 | 1.8 ± 0.15 | 4 ± 0 | 2.3 ± 0.1 | 5 ± 0.1 | 3.3 ± 0.45 |
| c16.1n9 | 0.2 ± 0.2 | 0.3 ± 0 | 0.3 ± 0.05 | 0.1 ± 0 | 0.1 ± 0 | 0.2 ± 0.1 |
| c18.1n7 | 2 ± 0.1 | 1.5 ± 0.15 | 3.5 ± 0.05 | 2.8 ± 0.1 | 5.8 ± 0.05 | 2.7 ± 0.1 |
| c20.1n11 | 2.6 ± 0.15 | 0 ± 0 | 0.4 ± 0.1 | 0.1 ± 0 | 0.3 ± 0.05 | 4.5 ± 0.85 |
| c20.1n9 | 1.7 ± 0.05 | 0.1 ± 0 | 1.1 ± 0.05 | 0.6 ± 0 | 0.6 ± 0.05 | 0.7 ± 0.1 |
| c20.1n7 | 2.1 ± 0.05 | 0 ± 0 | 0.6 ± 0.05 | 0.2 ± 0 | 0.7 ± 0.1 | 1.7 ± 0.2 |
| **Σ MUFA** | **11.3 ± 0.05** | **26.3 ± 0.15** | **12.7 ± 0.25** | **10.7 ± 0.8** | **15.3 ± 0** | **18.6 ± 0.85** |
| c18.2n6 | 0.8 ± 0.1 | 4 ± 0.25 | 1.9 ± 0.05 | 2.1 ± 0.3 | 1 ± 0 | 1.9 ± 0.65 |
| c18.3n3 | 1.2 ± 0.1 | 1.3 ± 0.05 | 2 ± 0.1 | 1.2 ± 0.45 | 0.5 ± 0 | 0.7 ± 0.15 |
| c18.4n1 | 1.3 ± 0.2 | 0.2 ± 0 | 2.9 ± 0.25 | 0.5 ± 0.3 | 1 ± 0.1 | 0.1 ± 0 |
| c20.2n6 | 0.6 ± 0.1 | 0 ± 0 | 0.6 ± 0.05 | 0.5 ± 0.05 | 0.3 ± 0.05 | 0.3 ± 0.1 |
| c20.4n6 | 1.4 ± 0.15 | 0.05 ± 0.05 | 1 ± 0.05 | 1.7 ± 0.05 | 1.9 ± 0.25 | 0.6 ± 0.15 |
| c20.5n3 | 10.5 ± 0.55 | 0.7 ± 0.05 | 11.8 ± 0.6 | 15.5 ± 0.4 | 19.4 ± 0.35 | 4.1 ± 2.3 |
| c22.5n3 | 1.7 ± 0.2 | 0.05 ± 0.05 | 0.5 ± 0.05 | 0.4 ± 0.1 | 0.4 ± 0.05 | 0.8 ± 0.25 |
| c22.6n3 | 13.2 ± 0.1 | 0.3 ± 0.1 | 12.1 ± 0.2 | 14.5 ± 0.4 | 6.8 ± 0 | 1.7 ± 0.8 |
| **Σ PUFA** | **32.1 ± 1.4** | **7 ± 0.35** | **34.1 ± 0** | **37.3 ± 0.7** | **32.4 ± 0.15** | **10.6 ± 3** |
| i15.0 | 0.1 ± 0 | 0.4 ± 0.05 | 0.6 ± 0.05 | 0.1 ± 0 | 0.3 ± 0 | 0.4 ± 0 |
| i17.0 | 2.9 ± 0.15 | 0.2 ± 0 | 0.5 ± 0 | 0.5 ± 0.05 | 0.8 ± 0.05 | 1 ± 0.05 |
| a17.0 | 1.1 ± 0.05 | 0.2 ± 0.05 | 0.1 ± 0 | 0.3 ± 0.05 | 0.3 ± 0 | 0.5 ± 0 |
| c17.0 | 1.4 ± 0.05 | 0.8 ± 0 | 1.2 ± 0.1 | 1.8 ± 0.05 | 1 ± 0.1 | 3.6 ± 0.1 |
| **Σ Odd** | **10 ± 0.45** | **5.4 ± 0.1** | **8.6 ± 0.3** | **6.8 ± 0.2** | **9.4 ± 0.25** | **11.7 ± 0.55** |
| **Σ EFA** | **2 ± 0** | **5.2 ± 0.3** | **3.9 ± 0.15** | **3.3 ± 0.75** | **1.5 ± 0** | **2.5 ± 0.8** |
| **Σ LCPUFA** | **25.1 ± 0.8** | **1 ± 0.1** | **24.9 ± 0.75** | **31.6 ± 0.75** | **28 ± 0.1** | **6.3 ± 3.25** |

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