AN ABSTRACT OF THE DISSERTATION OF

Katelyn Marie Bosley for the degree of Doctor of Philosophy in Fisheries Science presented on June 16, 2016

Title: An Integrated Approach to the Investigation of Age, Growth and Population Dynamics of Burrowing Thalassinidean Shrimps in a U.S. West Coast Estuary

Abstract approved:

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Two indigenous species of burrowing shrimp inhabit and often dominate the intertidal zone of estuaries along the US West Coast, the ghost shrimp, *Neotrypaea californiensis,* and the blue mud shrimp, *Upogebia pugettensis.* Both species are considered ecosystem engineers and play a role in maintaining estuarine health and ecosystem function. They also have a negative interaction with oyster production in Pacific Northwest (PNW) estuaries, which has necessitated a better understanding of their ecology and population dynamics in order to try to manage their impacts. Development of population models requires detailed information regarding an animal's age and growth. Because crustaceans do not retain calcified structures over a lifespan, estimates of age are typically based on body size which can vary significantly over environmental gradients. A series of field and laboratory studies were conducted to understand factors controlling growth of burrowing shrimps in the Yaquina River estuary and validate an alternative, biochemically-based (lipofuscin) aging technique as a method to overcome the challenges of determining age

in crustaceans. Analysis of shrimp diets along an estuarine gradient using fatty acid and stable isotope biomarkers revealed spatial patterns in diets between species and showed condition to be associated with availability of high-quality foods. Laboratory investigations of temperature on shrimp growth rates indicated temperature to be less important in controlling growth than food but may influence the accumulation of lipofuscin at environmental extremes for *N. californiensis*. Field growth experiments showed that lipofuscin accumulation rate in *N. californiensis* was consistent between sites but growth varied significantly. Size was a better predictor of age for *U. pugettensis* than lipofuscin concentration but growth was shown to correlate with thermal history. Finally, mortality rate was estimated for *N. californiensis* using the lipofuscin aging method and a cohort-based approach with data from 4 years of broad-scale population surveys in Yaquina Bay, Oregon. Mortality rate for adult *N. californiensis* was estimated to be 0.719 (95% CI; 0.633-0.793 yr^{-1}) and did not vary significantly across cohorts. Population simulations were also conducted to understand current and future patterns in shrimp density. Simulations revealed that spatial patterns in burrowing shrimp density could be explained by variation in mortality and recruitment rates. Results from these studies provide information needed to incorporate population ecology into management plans for burrowing shrimp in PNW estuaries. Methodologies developed in this project could also be applied to improve understanding of growth and population dynamics of other hard-to-age crustacean species on the US west coast and worldwide.

An Integrated Approach to the Investigation of Age, Growth and Population
Dynamics of Burrowing Thalassinidean Shrimps in a U.S. West Coast Estuary

by
Katelyn Marie Bosley

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I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes the release of my dissertation to any reader upon request.

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Katelyn Marie Bosley, Author

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CONTRIBUTION OF AUTHORS

Dr. Louise Copeman was involved in experimental design, laboratory analysis of tissue samples and writing of Chapter 2. Keith Bosley also provided technical guidance on experimental design and data analysis for Chapter 2. Dr. Tom Wainwright assisted with model development and interpretation for Chapter 4. Dr. Brett Dumbauld provided comments on Chapters 2-4 and provided long-term monitoring data for Chapter 4.

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DEDICATION

This dissertation is dedicated to my grandfather, Donald Shetler, who always encouraged me to think big.

CHAPTER 1

INTRODUCTION AND OVERVIEW

Quantification of age and growth dynamics is an important step in the development of population models and is essential information needed for management of biological resources. Many of the models that are currently used in population ecology are based on either size-structure or age-structure. Implementation of these structured models have posed a problem for researchers interested in understanding the population dynamics of crustaceans, which can exhibit significant variation in growth and have limited methods available for accurately determining age (reviewed in Hartnoll 1982). Crustaceans periodically molt and therefore do not retain hard structures containing evidence of their age like an otolith does in fish. As a result, aging methods for crustaceans have relied primarily on analysis of length-frequency data which is inherently imprecise because it does not take into account environmental variability that affects growth rate and maximum size (Fonseca & Sheehy 2007, Bosley & Dumbauld 2011). Numerous studies have shown growth patterns in crustaceans vary with environmental variables, such as food, temperature and salinity (Hartnoll 1982, Oh & Hartnoll 2000, Paglianti & Gherardi 2004, Fockedey et al. 2005, Brylawski & Miller 2006, Hufnagl & Temming 2011, Stoner et al. 2013) but few have attempted to incorporate environmental variability into population models.

Researchers have begun to explore alternative methods of age determination to overcome the challenges in assessing crustaceans age based on body-size measurements and analysis of the biochemically-produced "aging pigment", lipofuscin, has emerged as a viable method (Sheehy et al. 1990, Sheehy & Prior 2008, Puckett et al. 2008, Bosley & Dumbauld 2011). Lipofuscin accumulates in tissues as a by-product of intra-cellular lipid peroxidation (Terman & Brunk 1998, 2004), and is retained in post-mitotic cells over a lifespan (Sheehy & Ettershank 1988, Terman & Brunk 1998, 2004). Intracellular concentration of the pigment increases with time and has been used to assess age in a number of different crustacean species including the European lobster, *Homarus*

gammarus (O'Donovan & Tully 1996, Sheehy & Bannister 2002), American lobster, *Homarus americanus* (Wahle et al. 1996), tiger prawns, *Penaus monodon* and *Penaeus japonicaus* (Sheehy et al. 1995, Vila et al. 2000), polar amphipods, *Waldeckia obesa* and *Eurythenes gryllus* (Bluhm et al., 2001; Bluhm et al., 2001), the stomatopod, *Oratosquilla oratoria* (Kodama et al. 2006), Antarctic shrimp, *Notocrangon antarcticus* (Bluhm & Brey 2001), the crayfish species, *Cherax quadricarinatus* and *Pacifastacus leniusculus* (Sheehy et al. 1994, Belchier et al. 1998, Fonseca et al. 2005) and the blue crab, *Callinectes sapidus* (Ju et al. 1999, Puckett et al. 2008). However, there has been concern over its widespread application because the formation of lipofuscin is a metabolic process which is controlled by environmental variables in poikilotherms, specifically temperature (Sheehy et al. 1995, Sheehy 2002).

A study conducted by Bosley and Dumbauld (2011) used analysis of lipofuscin to estimate age and conduct demographic assessments for the burrowing shrimp, *Neotrypaea californiensis*, which inhabits intertidal sediments in Pacific Northwest (PNW) estuaries. The study found lipofuscin accumulation was consistent among spatially separated populations despite strong variation in size-at-age both across and within populations. Estimation of age structure with analysis of lipofuscin was an improvement over previous studies that had described population dynamics of burrowing shrimps on the West Coast using length-based methods (Bird 1982, Dumbauld et al. 1996, Feldman et al. 2000). Using analysis of lipofuscin, Bosley and Dumbauld (2011) provided new information regarding longevity of the species, which they determined to be more than twice the previous estimate (Dumbauld et al. 1996). The study also provided evidence that lipofuscin aging could be used to acquire more accurate estimates of population parameters (i.e., mortality, recruitment, age-structure) for burrowing shrimp that are of ecological and economic importance.

The burrowing shrimps, *N. californiensis* and *U. pugettensis*, are important components of estuaries in the Pacific Northwest (PNW). They inhabit vast expanses of intertidal mudflats and are considered as "ecosystem engineers" because they facilitate biogeochemical processes within estuarine sediments and influence community

composition of benthic fauna (Posey 1986, Posey et al. 1991, Berkenbusch & Rowden 2003, Berkenbusch et al. 2007, Ferraro & Cole 2007, D'Andrea & Dewitt 2009). As climate change threatens the long-term sustainability of estuarine communities, there is a need to understand more about how estuarine species interact with their environment and their role in the food web (Kennedy 1990, Kaustuv et al. 2001, Kennedy et al. 2002, Barbier et al. 2014). Development of methods to assess shrimp populations and identification of factors contributing to their growth and survival is an important first step in application of conservation and management frameworks.

Burrowing shrimps endemic to PNW estuaries are also of significant interest because they act as pest species to the shellfish aquaculture practices in the region (Dumbauld et al. 2006). Commercial aquaculture in the PNW is a multi-million dollar industry and in 2014 accounted for 40% of the nation's total shellfish production (Voorhees 2014). The revenue generated through aquaculture supports many small businesses and sustains a shellfish farming culture that has spanned many generations. These shrimp threaten shellfish and particularly oyster production when material ejected onto surface sediments from burrowing activities bury young animals causing them to die. This interaction has been a concern to growers for over 50 years and until recently shellfish farmers have used topical pesticides to control shrimp on oyster beds (Feldman et al. 2000, Dumbauld et al. 2006). Concern over the environmental impacts of using chemical methods of control shrimp has forced the industry to consider alternatives (Chew 2002, Doughton 2015). Currently the industry is working to develop an integrated pest management (IPM) framework with the goal of achieving economically and ecologically sustainable shrimp management (Dumbauld et al. 2006) and a large part of achieving successful IPM is having the ability to predict changes in shrimp populations.

With both ecological and economic incentives focusing research on burrowing shrimp, this project was designed to advance understanding of age, growth, and population dynamics in *N. californiensis* and *U. pugettensis* using an integrated approach. The research combines numerous methodologies, including biochemical analysis, field and laboratory studies, population surveys and numerical modeling to understand growth

variability in burrowing shrimp populations and confirm applicability of the lipofuscin aging method for future population studies and management.

The primary objectives of this dissertation research were:
1. Determine spatial variability in food resources using stable isotope and fatty acid trophic biomarkers for both *N. californiensis* and *U. pugettensis*
2. Validate the lipofuscin aging method for burrowing shrimp and determine how environmental influences affect growth and lipofuscin accumulation rate in burrowing shrimp in Yaquina Bay, Oregon
3. Apply the lipofuscin aging method to conduct broad scale demographic assessment and estimate mortality rate for *N. californiensis* in Yaquina Bay, Oregon

Previous studies have shown burrowing shrimp exhibit spatial patterns in growth and abundance (Bird 1982, Bosley & Dumbauld 2011, Dumbauld unpublished, D'Andrea unpublished). The study presented in Chapter 2 sought to test the hypothesis of spatial variability in food quality/quantity within a dynamic estuarine ecosystem. To date, limited work has been done to resolve the diets of these two species and this work provides the first explanation of how *N. californiensis* and *U. pugettensis* feed and grow in a spatially heterogeneous estuarine environment. While it has been well documented that food quality and temperature influence growth in crustaceans (Hartnoll 1982, Oh & Hartnoll 2000, Paglianti & Gherardi 2004, Fockedey et al. 2005, Brylawski & Miller 2006, Hufnagl & Temming 2011, Stoner et al. 2013), broad scale application of the lipofuscin aging method requires knowledge of how those factors control accumulation of the aging pigment. The work presented in Chapter 3 sought to identify how environmental parameters (i.e. food, temperature) control growth and lipofuscin accumulation rate in the burrowing shrimp *N. californiensis* and *U. pugettensis* and validate lipofuscin as a robust alternative to body length measurements. The lipofuscin aging method has the ability to overcome challenges of traditional length-based methods

of age determination and has great potential for applying structured population models to species where age is not well correlated to size. In Chapter 4, lipofuscin based aging was used to estimate population parameters for *N. californiensis*. Mortality rate was empirically derived and used to develop population scenarios relevant to ecology and management. Together these studies represent the "next generation" of population studies for burrowing shrimp on the US West Coast and develops an understanding of burrowing shrimp ecology that can be used to guide future management in Yaquina Bay and other PNW estuaries where shellfish aquaculture occurs.

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CHAPTER 2

**IDENTIFICATION OF BURROWING SHRIMP FOOD SOURCES
ALONG AN ESTUARINE GRADIENT USING FATTY ACID ANALYSIS
AND STABLE ISOTOPE RATIOS**

ABSTRACT

Two species of burrowing shrimp occur in high densities in U.S. West Coast estuaries, the ghost shrimp, *Neotrypaea californiensis*, and the blue mud shrimp, *Upogebia pugettensis*. Both species of shrimp are considered ecosystem engineers as they bioturbate and irrigate extensive galleries within the sediment. While their burrows comprise a dominant habitat type in west coast estuaries, little is known about these shrimps' diet and their role in estuarine food webs. The primary goals of this study were to identify major components of burrowing shrimp diets and detect variation in these diets along an estuarine gradient using combined fatty acid (FA) and stable isotope (SI) analyses. Shrimp and potential food sources including eelgrass blades, epiphytes, *Ulva*, sedimentary particulate organic matter (SPOM), burrow walls and suspended particulate organic material (POM) were sampled at different locations within Yaquina Bay, Oregon, in August 2012. Both SI and FA analyses indicated differences in food ingested by shrimp along the estuarine gradient. SI values showed that diets for *U. pugettensis* consisted of carbon sources derived primarily from POM and SPOM while POM and epiphytes were primary carbon sources for *N. californiensis*. Shrimp from lower estuarine sites had high levels of 16:1ω7 and 20:5ω3 FAs suggesting their diet is enriched with marine diatoms. Shrimp from upriver showed greater proportion of FA associated with dinoflagellates and terrestrial sources as indicated by a high percentage of C_{18} polyunsaturated FAs (PUFAs). This is the first study to evaluate diets of these two shrimp species using complimentary FA and SI approaches.

INTRODUCTION

Two indigenous species of burrowing shrimp inhabit and often dominate the intertidal zone of estuaries along the US West Coast, the ghost shrimp, *Neotrypaea californiensis,* and the blue mud shrimp, *Upogebia pugettensis.* Both species are important components of estuarine ecosystems and are considered ecosystem engineers because they influence the benthic macrofaunal and microfaunal community and provide a number of services including oxygenation of sediments, enhancement of nutrient flux and remineralization through their burrowing activity (Berkenbusch and Rowden 2003; Howe et al. 2004; D'Andrea and Dewitt 2009). They are important components of the estuarine food web both as grazers/suspension feeders and as prey for larger secondary consumers (Dumbauld et al. 2008).

Availability of high quality food resources is an important factor controlling crustacean growth and survival (Hartnoll 2001). Bosley and Dumbauld (2011) observed differences in average body size among spatially separated populations of *N. californiensis* and hypothesized that food quality and availability could be important in limiting growth rates. Previous work on burrow morphology suggest that *N. californiensis* is a motile deposit feeder that utilizes detritus and microorganisms buried in estuarine sediments (MacGinite 1934, Griffis & Suchanek 1991, Nickell & Atkinson 1995). Studies of other Axiidean shrimp that inhabit estuaries in Japan, Australia and South Africa however, have shown that this taxonomic group once thought to be exclusively deposit feeders based on burrow morphology and mouthpart morphology may feed opportunistically and have broader diets (Boon et al. 1997, Shimoda et al. 2007, Antonio and Richoux 2014). Carbon sources have been examined for *N. californiensis* in estuarine food web studies (Kwak & Zedler 1997, Page 1997), but these studies took place in estuaries near the southern end of the species' range and did not address spatial patterns in diet. To date, limited studies have been done to confirm dietary sources for *N. californiensis* (Kwak & Zedler 1997) but none have determined how spatial variation in nutrition may influence their demography.

Both burrow structure and the morphology of feeding appendages in *U. pugettensis* indicate they are suspension feeders (Griffis & Suchanek 1991, Nickell & Atkinson 1995) and they are effective at removing particles from the overlying water column (Griffen et al. 2004). Trophic studies on related Gebiidean mud shrimps demonstrated that they may also farm microorganisms on the burrow wall and even ingest the burrow lining as an additional source of nutrition (Coelho et al. 2000; Daniel Abed-Navandi et al. 2005). It has been hypothesized that like its congeners *Upogebia major* and *Upogebia yokoyai* in Japan (Kinoshita et al. 2003; Kinoshita et al. 2008), *U. pugettensis* can employ multiple feeding strategies and feed directly on organisms in the burrow wall, but no work has been done to support the hypothesis or determine if feeding mode varies spatially.

Fatty acids (FA) are major constituents of lipids, which are the densest form of energy for plants and animals (Sargent 1976). Some FAs are specific to certain primary producers and conservatively transferred to and concentrated in upper level consumers through their diets, making them suitable for use as biomarkers (Parrish et al. 2000). Previous studies used FAs as biomarkers to identify bacteria (Rajendran et al. 1992), diatoms (Parrish et al. 2000), dinoflagellates (Parrish et al. 2000), macroalgae (Wahbeh 1997), zooplankton (Falk-Petersen et al. 2002) and seagrasses (De Leeuw et al. 1995) in the diets of a number of species. These biomarkers can be used to trace the origin and movement of organic matter through a food web. Because they are integrated over a longer time scale than gut content analyses, they provide integrated dietary information that does not over-emphasize prey with indigestible hard body parts (Kharlamenko et al. 2001, Parrish 2013).

Stable isotope (SI) ratios also provide time-integrated information on animal feeding histories and are commonly applied in ecological studies. The stable isotopes of carbon and nitrogen can be evaluated to provide information regarding source of nutrition and trophic position. Carbon isotopes, ^{13}C and ^{12}C, are fixed in different ratios relative to each other by different primary producers and these ratios show little change when assimilated in consumer tissues (Fry & Sherr 1984). This allows the ratio of $^{13}C/^{12}C$,

expressed as $\delta^{13}C$ (with units of parts per thousand ‰), to be used as a tracer for sources carbon through a food web (Peterson & Fry 1987). Likewise, the trophic position of an organism can be estimated with the ratio of $^{15}N/^{14}N$, as $\delta^{15}N$ increases relative to the organism's diet (Peterson & Fry 1987; Post 2002; Vander Zaden & Rasmussen 2001). Combining SI ratios with FA composition data provides additional resolution of trophic relationships, especially if carbon sources vary as is often the case in estuarine systems (Coffin et al. 1994, Copeman et al 2009, Parrish 2013).

Complimentary FA and SI approaches have been used to identify diets of several benthic infaunal invertebrates including clams (Kharlamenko et al. 2001) and the burrowing shrimps *Trypaea australiensis* (Spilmont et al. 2009), *Pestarella tyrrhena* , *Corallianassa longiventris* (Abed-Navandi et al. 2005), and *Callichiruis kraussi*, and *Upogebia africana* (Antonio & Richoux 2014). In all cases this dual approach indicated that a significant portion of these shrimp's diet was derived from suspended particulate organic material (POM) along with plant and algal material including diatoms and benthic microalgae. These studies provide evidence that infaunal organisms exhibit plasticity in feeding mode depending on available food resources over various spatial and temporal scales (Alfaro et al. 2006; Antonio & Richoux 2014).

Estuaries are dynamic environments where primary production varies both spatially and temporally (Correll 1978, Cloern 1991, Cloern et al. 2014). This means that sedentary benthic consumers must be adapted to variation in the quality and quantity of available food. Burrowing shrimp populations are found across a wide spatial extent covering a range of physical, chemical and biological characteristics (MacGinite 1934, Macginitie 1935, Bird 1982, Swinbanks & Luternauer 1987, DeWitt et al. 2004). Given the diversity in food quality throughout estuaries, we hypothesize that burrowing shrimp likely developed feeding strategies to maximize nutrition in this variable trophic environment.

In this study, we used the complimentary approach of FA and SI ratios to identify and contrast the major dietary components of two co-occurring species of burrowing shrimp and determine whether food sources varied spatially across a gradient in the ocean

dominated coastal plain estuary, Yaquina Bay, Oregon, USA. We test they hypothesis that food sources vary between species and within species along the estuarine gradient. We also used percent organic material and total FA to determine spatial variability in shrimp condition and make inferences about the effects of food quality on growth and population demographics in a changing climate.

MATERIALS AND METHODS

Study area and sample collection

Samples were collected from Yaquina Bay on the central coast of Oregon, USA (44°37' 08.07''N, 124° 02' 29.68''W). The bay is relatively small, encompassing approximately 17 km^2 at mean high water (Shirzad et al. 1988, Fig 2.1) with ~ 5.21 km^2 of intertidal area (Young et al. 2015). Yaquina Bay varies between a partially-mixed and well-mixed estuary depending on the season (Burt & McAlister 1959). Two species of eelgrass (*Zostera marina* and *Z. japonica*) are found throughout the bay with an aerial survey showing *Z. marina* covering ~18.7% of the tidalflat and benthic macroalgae covering ~2.9% (Young et al. 2015).

Samples of shrimp and potential food items were collected during the summer growing season when productivity is at a peak. Food items sampled included eelgrass (*Z. marina*), epiphytic algae, suspended particulate organic matter from the water column (POM), and sediment particulate organic matter (SPOM) from sediment surface (top 2 mm), sediment cores (1-10 cm depth) and burrow walls (for *U. pugettensis* only). *N. californiensis* and *U. pugettensis* were each collected from three different locations along the estuarine gradient (Fig 2.1) using a shrimp pump. SPOM samples were collected at the same locations as shrimp. Additional food web samples were collected from Seawall Island (SWI), Idaho Flats (IF), Raccoon Flats (RAC), Upper River (UPR) and Tongue Island (TI) locations representing an estuarine gradient, with the exception of eelgrass and epiphytes, which were not collected at IF. The RAC location was used as the midriver site for eelgrass and epiphytes.

POM samples were collected at shrimp sites during high tide with a 60 μm mesh plankton net which was towed horizontally for 3 minutes. Pieces of eelgrass and macroalgae found in POM samples were carefully removed prior to freezing and storage at -80°C. Epiphytes were gently scraped from eelgrass blades with a clean scalpel and the clean eelgrass blades were then used for FA and SI analysis. Surface SPOM samples were collected by scraping a 10 cm^2 area of the sediment surface to 2 mm depth with a clean spatula. Sediment cores were 3 cm in diameter and were taken to a depth of 10 cm within the area where sediment surface was removed. Burrow wall samples for *U. pugettensis* were collected by excavating burrows with a shovel and scraping the burrow lining to a depth of 1.5 mm with a clean spatula. Burrow lining samples were collected within 20 cm of the surface openings. All samples, except SPOM samples, were rinsed with de-ionized water prior to freezing (-80°C) and stored for less than one year prior to lipid and SI analysis.

A total of 5 samples were taken for all diet items at each location, excluding POM. Two of these samples were used for FA analysis and three for SI analysis. Two POM samples were collected for each location and these samples were homogenized and then divided for analysis of FA and SI ratios. Where possible, twenty shrimp of each species were collected from each location, ten each for FA and SI analyses. Burrowing shrimp were kept alive in filtered marine seawater for a minimum of 24 hours to depurate prior to storage. All samples were collected within a two week period, August 21 –Sept 5, 2012.

Sample preparation and processing

Shrimp were sexed and wet weights taken prior to analysis. *U. pugettensis* were large enough to be bisected along the dorsoventral axis so one half of the animal was used for FA analysis and the other for SI analysis. Shrimp percent organic matter content was determined using a weight loss-on-ignition (LOI) method where portions of whole body shrimp homogenate were dried, mass measured, then placed in a muffle furnace (550° C for 4 hours) to remove organic material. Samples were reweighed and % organic

matter was calculated as the difference in pre- and post- combustion weights. Percent organic matter of SPOM was also determined using LOI methods described above. Chlorophyll *a* and phaeopigment concentration were determined for SPOM with spectrophotometric methods according to Lorenzen (1967).

For analysis of δ ^{13}C and δ ^{15}N SI ratios, all samples were washed with distilled water, dried at 60 °C and ground to a fine powder using a mortar and pestle. Epiphytes, POM, and sediments were treated with 1N HCl to remove carbonates prior to analysis. Samples were then sent to the Colorado Plateau Stable Isotope Laboratory at Northern Arizona University in Flagstaff, Arizona (CPSIL-NAU) for analysis of δ^{13}C, δ^{15}N, and C:N ratios. Samples were run on a Thermo-Electron Delta V Advantage IRMS, configured through a Finnigan CONFLO III for automated continuous-flow analysis of δ^{15}N and δ^{13}C, using a Carlo Erba NC2100 elemental analyzer. The analytical precision was based on the standard deviation of replicates from an internal standard and was 0.04‰ for δ^{13}C and 0.06‰ for δ^{15}N. Stable isotope ratios were expressed in the δ unit notation calculated using the following equation: δX (‰) $= [(R_{sample}/R_{standard}) - 1] \times 10^3$ where X represents ^{13}C and ^{15}N and the R is the ^{13}C/^{12}C or ^{15}N/^{14}N ratio respectively.

Fatty acid methyl esters (FAMEs) were derivatized from burrowing shrimp tissue using a direct trans-methylation procedure where lipids were extracted and FAs esterified in a single step (Parrish et al 2014). Briefly, shrimp tissues were prepared by rinsing with distilled water and then homogenized. A 40-50 mg aliquot of homogenate was measured out into a 10 ml culture tube with 3 ml of methylating solution (MeOH:HCL:CH$_2$CL$_2$, 10:1:1). Samples were then placed in a heating block at 85 °C for 2 hours. After cooling, 1.5 ml of Nanopure water was added in addition to 1.5 ml of 23:0 internal standard in hexane and 0.3 ml of CH$_2$Cl$_2$. Samples were vortexed then centrifuged. The upper hexane layer was transferred to a 1.5 ml glass vial, evaporated to dryness under nitrogen and re-suspended with 1 ml of hexane for analysis by gas chromatography.

Lipid extractions for all other food web samples followed procedures outlined by Parrish (1999) using a modified Folch procedure (Folch et al. 1957). Lipid extracts were derivatized using methods outlined by Copeman et al. (2015). Briefly, FAMEs were

trans-esterified using the Hilditch reagent (0.5 N H_2SO_4 in anhydrous MeOH) for 1 hour at 100 °C as described by Budge et al. (2006). The FAMEs were then analyzed on an HP 7890 GC FID equipped with an autosampler and a DB wax+ GC column (Agilent Technologies, Inc., U.S.A.). The column was 30 m in length, with an internal diameter of 0.25 μm. The column temperature began at 65 °C and held this temperature for 0.5 min. Temperature was increased to 195 °C (40 °C min^{-1}), held for 15 min then increased again (2 °C min^{-1}) to a final temperature of 220 °C. Final temperature was held for 1 min. The carrier gas was hydrogen, flowing at a rate of 2 ml min^{-1}. Injector temperature was set at 250 °C and the detector temperature was constant at 250 °C. Peaks were identified using retention times based upon standards purchased from Supelco (37 component FAME, BAME, PUFA 1, PUFA 3). Nu-Check Prep GLC 487 quantitative FA mixed standard was used to develop correction factors for individual FAs. Chromatograms were integrated using Chem Station (version A.01.02, Agilent).

Statistical analysis

We estimated percent contribution of diet sources with SI mixing models for each species of burrowing shrimp and each location using the Stable Isotope Analysis in R (SIAR) package (Parnell et al. 2010). This model iteratively assesses the potential contributions of food sources to reflect the consumer SI ratios using a Bayesian context. The SIAR model provides a posterior probability distribution that describes the likelihood of a food source contributing to the consumer diet. Diet sources that did not differ significantly in their isotopic signatures were combined prior to the SIAR analysis. Within the SIAR model, trophic fractionation for all food sources was assumed to be 0.75 ± 0.25 for $\delta^{13}C$ and 2.5 ± 1.5 for $\delta^{15}N$, respectively (Vander Zanden & Rasmussen 2001, Post 2002, Yokoyama et al. 2005). The trophic fractionation for $\delta^{15}N$ was selected because it includes the likely range of fractionation values for primarily herbivorous marine invertebrates (Vander Zanden & Rasmussen 2001, Vanderklift & Ponsard 2003, Yokoyama et al. 2005). The model was run independently for each location with isotopic ratios for shrimp and diet items collected at that single location. The only exception was

for IF shrimps where isotope values for eelgrass and epiphyte were from the nearby RAC location because eelgrass and epiphytes were not collected at the IF sites.

Analysis of Variance (ANOVA) was used to examine the effect of location and species on isotopic ratios (δ^{13}C and δ^{15}N), % organic material and total FA (μg mg^{-1}) of shrimp. Because shrimp of both species were not sampled from the same location, one-way ANOVA with Tukey's pairwise comparisons by site were used to compare both among sites and between species. Sediment chlorophyll *a* (μg mg^{-1}), phaeopigment (μg mg^{-1}) and % organic material were also compared via ANOVA and tested for differences among sites for each sample type (scrape, core, burrow lining).

Statistical differences in FAME profiles were determined using non-parametric, multivariate tests on untransformed percent total FA data. For each sample types, FAMEs that had a maximum value less than 1% of total FAs in at least one sample were removed prior to analysis. Differences in FA profiles between sites were examined with non-metric multidimensional scaling (NMDS) ordination procedures using a Bray-Curtis distance measure. The NMDS ordination procedure allows visualization of multivariate data structure and pairwise dissimilarity between sample units in a low-dimensional space (McCune & Grace 2002). Additional tests of similarity were done using non-parametric multi-response permutation procedure (MRPP) and permutational multivariate ANOVA (PerMANOVA) to quantify mean differences in the FAME profiles among species and sites. FA biomarkers were correlated to NMDS axes to identify likely food sources for shrimp from different sites and determine which FAMEs were important in driving differences in shrimp FA profiles. All analyses were done in the statistical software package R (R core development team, 2013).

Simple linear regression was used to describe the relationship between burrowing shrimp FA biomarker values and % organic matter to identify diet sources that contributed to differences in shrimp condition. In addition, the relationship between FA biomarkers and δ^{13}C values for POM and SPOM was used to identify composition of these samples which are typically comprised of a mixture of sources (Page 1997, Lowe et al. 2014).

RESULTS

Stable isotope ratios of shrimp and diet items

Stable isotope values for food sources showed considerable spatial variation throughout the estuary, within and among sample types. With the exception of *N. californiensis* at the IF location, shrimp δ^{15}N values were relatively uniform (~10 ‰) between species and across sites (Fig. 2.2). *N. californiensis* sampled from IF were more enriched in ^{15}N than shrimp from other locations sampled in the bay with a mean δ^{15}N value 1.42 ‰ greater than all other sites. *U. pugettensis* from the mid-river site (RAC) were slightly more depleted in ^{15}N than shrimp from the other locations (Fig 2.2, Table 2.1). Shrimp from different locations within the bay were significantly different from each other with respect to δ^{13}C. Carbon isotope values exhibited a strong spatial trend with shrimp located near the estuary mouth being more enriched in δ^{13}C than those collected from sites up river. *N. californiensis* had the greatest variation in δ^{13}C across space with IF shrimp being most enriched (δ^{13}C = -14.66 ± 0.31 ‰) and the upriver TI site being most depleted (δ^{13}C = -22.02 ± 0.13 ‰, Fig 2.2, Table 2.1). SI ratios of potential food items showed spatial trends similar to those observed in the shrimp. In general, δ^{13}C values differed by food type with eelgrass being most enriched and POM being most depleted (Tables 2.2 & 2.3). Epiphytes and *Ulva* were enriched in both δ^{13}C and δ^{15}N at mid river locations (Tables 2.3 & 2.4). δ^{15}N ratios also varied slightly among food types with values ranging from a low of 6.39 ± 0.80 ‰ for *Ulva* at the SWI location to a high of 9.30 ± 0.06 ‰ for POM at the most upriver location, TI (Tables 2.2 & 2.4). Apart from significantly higher POM δ^{15}N at TI, diet items represented a single trophic level of primary producers. SPOM samples were more depleted in ^{13}C and ^{15}N than other sampled food sources. However, both sediment scrapes and core isotope values at the IF site where *N. californiensis* were sampled showed significant enrichment relative to sediment scrapes and cores collected from the other locations in the estuary (Tables 2.5 & 2.6). Sediment scrapes, cores and linings (for *U. pugettensis* only) within a site did not differ significantly with respect to δ^{13}C and δ^{15}N values and were therefore combined

into a single "SPOM" category for SIAR analysis of shrimp diet at each location (Tables 2.5 & 2.6).

The five source SIAR mixing models provided estimates of percent contribution of the various potential diet sources and indicated differences in the relative contribution of food sources to diet between species and within species across space. The model showed POM as a primary contributor to diets of *N. californiensis* making up an average of 30 – 45% of the diets across all three locations (Fig 2.3). There was little overlap in the probability distribution of POM and other food sources for SWI and TI suggesting material in POM to comprise the main food source at these locations. The models showed diets at these sites were made up of a mixture of eelgrass, epiphyte and *Ulva* sources, with SPOM and epiphytes being slightly more important at SWI and TI, respectively (Fig 2.3). SIAR models indicated diets at IF were comprised of a mixture of POM, epiphytes and *Ulva* while SPOM and eelgrass on average made up < 10 % of shrimp diets (Fig 2.3).

Diets of *U. pugettensis* in the lower estuary were comprised of a mixture of epiphyte (~24%), POM (~30%), and SPOM (~23%) sources (Fig 2.3). Diets in the lower estuary sites, IF and RAC, were nearly identical with approximately equal contribution from the primary sources. The upriver location (UPR) showed the highest contribution of POM (~ 46%) with a mixture of epiphyte (~ 20%) and SPOM (~ 25%) sources also being important. Neither *Ulva* nor eelgrass sources were important components of diets for *U. pugettensis* at the upriver location and combined made up an average < 9 % of shrimp diets (Fig 2.3).

FAMEs in shrimp and their food sources

A total of 18 FAs were detected at > 1% of the total FAs in shrimp tissues (Table 2.7). Shrimp of both species were generally high in 20:5ω3 (EPA, ~25%). They also had relatively high proportions of saturated FAs (SFA) 14:0, 16:0; monounsaturated FAs (MUFA) 16:1ω7, 18:1ω7, 18:1ω9; and polyunsaturated FAs (PUFA) 18:4ω3, 22:6ω3. All of the major FAs detected in shrimp varied between species and among locations within a species. In general, *U. pugettensis* was more elevated in eicosapentaenoic acid

(EPA, 20:5ω3) and 14:0 than *N. californiensis* while *N. californiensis* had higher proportions of 16:1ω7 and 18:1ω9. There were many FAs that varied by site within a species. Proportions of EPA and 16:1ω7 for both species were highest at sites near the estuary mouth (Table 2.7) while proportions of 16:0, 18:2ω6, 18:3ω3, and docosahexaenoic acid (DHA, 22:6ω3) were elevated at upriver sites. FA profiles for both species were comprised of similar amounts of SFA with a significantly greater proportion in samples from upriver locations (Table 2.7). *N. californiensis* had higher proportions of MUFA and significantly lower proportions of PUFA than *U. pugettensis* (post hoc Tukey tests, p >0.5). There was a strong spatial trend in the DHA/EPA ratios with significantly higher values upriver locations with values of 0.37 ± 0.03 (UPR) and 0.82 ± 0.09 (TI) for *U. pugettensis* and *N. californiensis* respectively.

Comparison of FA profiles for the shrimp showed significant differences across sites (perMANOVA, $F_{5,54} = 79.148$, $p < 0.001$ on 1000 permutations). Cluster of mean dissimilarities among sites showed differences between species (Fig 2.4). FA profiles of *U. pugettensis* from low and mid-river sites (IF and RAC) were more similar to each other than the UPR location. Likewise, *N. californiensis* from the low and mid river sites (SWI and IF) made up another cluster and the upriver TI location was most dissimilar from all other locations sampled. Within species comparisons of FA profiles showed strong spatial differences. NMDS ordination of FAs from *N. californiensis* resulted in a two-dimensional solution (stress = 0.133, $r^2 = 0.940$, 2.5a) revealing three distinct groups that corresponded to sites. All FA biomarkers (Table 2.8) correlated to the NMDS axes creating vectors with r^2 greater than 0.80 (Fig 2.5a, Table A1 in the appendix). The upriver TI location showed association with eelgrass and dinoflagellate biomarkers. FA scores showed that separation of the TI location was driven by high amounts of 16:3ω4, 18:3ω3, 18:4ω3, 20:4ω3 and 22:6ω3. Separation of the middle estuary site (IF) was mostly driven by bacterial and algal biomarkers. Lower river *N. californiensis* were strongly associated with diatom markers and higher levels of 16:4ω1, 20:4ω6 and 20:5ω3 FAs. Total FAs (μg mg^{-1} wwt) significantly correlated with NMDS axes showing increased values in SWI and TI shrimp (Table 2.8).

A two-dimensional ordination of FAMEs from *U. pugettensis* (stress = 0.154, r^2 = 0.926, Fig 2.5b) showed overlap in the IF and RAC locations which agreed with SIAR model output. These sites showed strong association with both diatom biomarkers (Fig 2.5b). Difference in upriver *U. pugettensis* was mainly driven by dinoflagellate and eelgrass biomarkers and shrimp from this location were associated with higher levels of 18:0, 18:2ω6 and 22:6ω3. Total FAs, bacterial biomarkers and algal markers were less important in discriminating among locations for *U. pugettensis* than in discriminations for *N. californiensis* (Table A1).

Shrimp condition indices

The amount of total FA per wet weight of shrimp (ug mg^{-1}) varied by location (Table 2.3, Fig 2.6a, ANOVA, $F_{5,54}$ = 37.87, p > 0.001). FA concentration was most variable in *N. californiensis* with this species having significant differences in FAs across all sites (post hoc Tukey tests, p < 0.05). SWI shrimp had significantly higher concentrations of FAs (82.21 ± 16.78 μg mg^{-1}) than shrimp collected at any other location within the bay. FA content in *N. californiensis* was lowest at the IF site (13.37 ± 7.00 μg mg^{-1}) and intermediate at the upriver TI location (34.50 ±15.63 μg mg^{-1}). FA concentration in *U. pugettensis* was more uniform and was not significantly different across sites (17.39 ± 7.91 to 30.34 ± 17.39 μg mg^{-1}, post hoc Tukey tests, p > 0.5).

Percent organic matter, which is often used as a proxy for body condition in shrimp, mirrored results observed in total FA per wet weight (Fig 2.6b). *N. californiensis* was more variable in % organic matter with SWI having the highest % organic content (82.57 ± 3.13%) and IF having the lowest (69.63 ± 2.92%). The downriver SWI site and upriver TI site were not significantly different with respect to tissue % organic matter (post hoc Tukey tests, p >0.5). Organic matter content in *U. pugettensis* was not significantly different across sites and was intermediate relative to the range of values for *N. californiensis* (72.05 ± 4.12 to 75.83 ± 4.07 μg mg^{-1} wwt, post hoc Tukey tests, p > 0.5).

Three significant relationships were found between % organic matter and selected FA biomarkers for *N. californiensis* and two for *U. pugettensis* (Fig 2.7). Bacterial and green algae biomarkers had significant negative correlations to body condition for *N. californiensis* (Fig 2.7a and 2.7b, bacterial; β = -8.20, t_{28} = -8.716, $p < 0.001$, adj r^2 = 0.721; chlorophyte; β = -18.11, t_{28} = -7.194, $p < 0.001$, adj r^2 = 0.65). Bacterial biomarkers showed a weak negative correlation with *U. pugettensis* condition and accounted for only 18% of the variation in data (Fig 2.7e, β = -6.046, t_{28} = -2.717, $p < 0.011$, adj r^2 = 0.180). Conversely, Rhodophyte biomarkers found in shrimp tissues were positively correlated with condition for both *N. californiensis* and *U. pugettensis*, explaining 55.8 % and 33.4% of the variation in % organic material respectively *(*Fig 2.7c and 2.7f, *N. californiensis*; β = 0.51, $t_{1,28}$ = 6.14, $p < 0.001$; *U. pugettensis*; β = 0.47, $t_{1,28}$ = 3.943, $p < 0.001$) .

Sediment characteristics

Chlorophyll *a* concentration in sediment samples varied across locations and by sample type, ranging from 24.08± 2.83 mg g^{-1} to 99.46 ± 6.90 mg g^{-1} (Table 2.9). Chlorophyll *a* concentrations for surface scrape and core samples were significantly different across all sites (scrape; $p < 0.001$, $F_{5,18}$ = 17.43, Core; $p < 0.001$, $F_{5,18}$ = 17.42). Highest values were seen at mid river locations IF and RAC (Table 2.9). Phaeopigment concentration in sediment scrapes and cores were also significantly different among sites (Scrape; $p < 0.001$, $F_{5,18}$ = 20.51, Core; $p < 0.001$, $F_{5,18}$ = 22.86) with highest values found at RAC (Table 2.9). Phaeopigments were lowest in core samples from the upriver, TI location. Organic matter within sediment scrapes and cores varied between sites (Scrape; $p < 0.001$, $F_{5,6}$ = 89.61, Core; $p < 0.001$, $F_{5,16}$ = 186.5). Organic matter was generally low in sediments ($< 8\%$) but elevated in samples from mud shrimp sites relative to ghost shrimp sites. Pigments were not significantly different in burrow linings (Chl *a*; p = 0.538, $F_{2,9}$ = 4.117, Phaeo; p = 0.523, $F_{2,9}$ = 0.697). However we did detect a difference in organic matter in burrow linings among sites ($p < 0.004$, $F_{2,3}$ = 52.83) with the highest levels seen at the mid-river RAC location (Table 2.9).

DISCUSSION

Using the complementary FA and SI approach to assessing burrowing shrimp diets, our data supported the hypothesis that the diets of *N. californiensis* and *U. pugettensis* vary from each other and tend to reflect the spatial heterogeneity of food sources available within the Yaquina River estuary. While both species appeared to feed on the same general mix of food sources, the relative contribution of those food sources differed and could explain spatial variation in shrimp condition within the estuary.

Spatial variation in burrowing shrimp diets
Ghost shrimp *(Neotrypaea californiensis)*

Based on burrow morphology and lifestyle, ghost shrimp, *N. californiensis*, are recognized as deposit feeders (Griffis & Suchanek 1991, Nickell & Atkinson 1995) yet the primary sources of their nutrition were not previously described. High levels of diatom markers in *N. californiensis* and relatively low DHA/EPA ratios confirmed diatoms are one of their primary food sources (Budge & Parrish 1998, Pepin et al. 2011, Lowe et al. 2014). Consistent with findings in other estuarine infaunal species, *N. californiensis* is heavily reliant on POM carbon obtained from overlying water column (Alfaro et al. 2006, Antonio & Richoux 2014).

Spatial variation in ghost shrimp diets was evident across the three areas sampled using both SI and FA. The SIAR models and FA biomarkers both indicated that *N. californiensis* in the lower estuary fed primarily on POM comprised of marine sourced diatoms further evident by the enriched ^{13}C values of lower estuary POM samples (δ13C ~ 21‰) (Peterson et al. 1985, Michener & Schnell 1994). Upriver shrimps also rely on POM as their primary nutritional source, however, the composition of POM upriver was dominated by dinoflagellates as indicated by relatively high DHA levels. The abundance of detrital material in POM samples is correlated with SFA:MUFA ratio, with higher concentrations of SFA found in detritus rich samples (Lowe et al. 2014). Our POM samples from the TI site were also high in SFA relative to MUFA, indicating relatively

high amounts of detrital matter in upriver samples. Depleted ^{13}C values combined with relatively high 18:2ω6 and 18:3ω3 FA suggest this material is terrestrial in origin (Table A1).

SI results suggest that *N. californiensis* from IF were more heavily dependent on marine macroalgae (*Ulva*) as a carbon source than shrimp collected from any other location within the bay (Fig 2.3). This is not surprising given that IF is a high intertidal site dominated by macroalgae during the late summer months when our sampling occurred (Kentula & DeWitt 2003). FA profiles of shrimp from IF showed strong association with chlorophyte, macrophyte and bacterial biomarkers confirming macroalgae as an important dietary carbon source. POM samples from the IF site were also rich in 20:4ω6, a FA which is associated with macroalgae (Galloway et al. 2012, Kelly & Scheibling 2012, Lowe et al. 2014). The SIAR model indicated epiphytes as important diet items for shrimp from IF. This finding agrees with other studies showing that epiphytes are important food source for benthic communities (Alfaro et al. 2006, Kasim & Mukai 2006, Belicka et al. 2012). Epiphyte samples from IF and other locations were proportionally high in EPA which is considered a diatom indicator (Kelly & Scheibling 2012, Parrish 2013, Lowe et al. 2014). Although eelgrass was not sampled at IF, epiphytic diatoms in Yaquina Bay are not specific to *Zostera* and occur on *Ulva* in addition to other submerged aquatic vegetation (Main & McIntire 1974). It is likely that marine diatoms present on *Ulva* are consumed along with the macroalgae itself at this location.

Bulk isotope values from the mid-river *N. californiensis* suggest a diet rich in microbially processed algal material. Shrimp from IF were most enriched in ^{13}C (-14.7 ± 0.31) and bacterial FA markers. SPOM samples and *Ulva* were also enriched in δ^{13}C at this site. Isotopic ratios can be influenced by physical or chemical controls on the CO_2 system (Coffin et al. 1994). During times of net autotrophy, there may be less isotopic discrimination by primary producers as carbon is fixed from the dissolved inorganic carbon (DIC) pool leading primary producers to be isotopically heavy (Coffin et al. 1994). This may explain why *N. californiensis* from IF were heavy in ^{13}C compared to

other locations. In addition, the shrimp at IF were found to be enriched in ^{15}N. During microbial mineralization of organic matter in sediments the total amount of nitrogen is reduced and ^{14}N is preferentially lost which can result in the enrichment of decomposed organic matter (Liu et al. 2006). Caraco et al. (1998) suggested that consumption of this microbially-processed organic material may shift δ^{15}N values and consumption of the heterotrophic bacteria themselves may enrich ^{15}N values of consumers (Riera 1998). These factors may explain elevated ^{15}N values in sediments and shrimp at the IF location.

Sources of carbon for *N. californiensis* from the upriver TI location were comprised mainly of material from POM, SPOM and epiphytes. All diet items, including SPOM, from the upriver location were most depleted in ^{13}C carbon suggesting a contribution of terrestrial carbon from upland plants to the diet (Fry & Sherr 1984, Peterson & Fry 1987, Michener & Schnell 1994). While it is difficult to distinguish from marine vascular and aquatic plants within an estuary using FA signatures alone (Parrish et al. 2000, Copeman et al. 2009), δ^{13}C indicated POM from TI to be comprised of terrestrial material which is consistent with the FA biomarker data for shrimp at that location. The proportion of SFA to MUFA at TI was very high indicating POM to be rich in detrital material which may also explain the mixed diet for shrimp living upriver. Sediments at TI were characterized by low δ^{15}N signatures which is typical of sediments containing high amounts of terrigenous detrital material (Mariotti et al. 1984, Peterson et al. 1985, Thornton & McManus 1994, Deegan & Garritt 1997). FA profiles for TI ghost shrimp also showed strong associations with dinoflagellate biomarkers which can be explained by high levels of DHA in upriver POM.

Blue Mud Shrimp *(Upogebia pugettensis)*

The spatial extent of the *U. pugettensis* population in Yaquina Bay is smaller in area than its ghost shrimp cousin and mud shrimp are found at lower tidal elevations (DeWitt et al. 2004). Burrow morphology and mouthpart structure indicate that *U. pugettensis* are filter feeders and previous studies have shown these animals have a large capacity to remove particles from the overlying water column (Griffis & Suchanek 1991,

Nickell & Atkinson 1995, Griffen et al. 2004). It was previously suggested that *U. pugettensis* and other Upogebiid shrimps may switch feeding modes to deposit feeding (Coelho et al. 2000; Griffen et al. 2004) or even feed on material cemented to their burrow linings (Dworshak 1987, Dobbs & Guckert 1988, Nickell & Atkinson 1995, Miller et al. 1996). Data from our study supports the hypothesis of flexibility in feeding mode, but we were not able to determine with certainty if *U. pugettensis* feed directly on burrow linings. Percent carbon and percent organic material were generally elevated in burrow walls relative to surrounding sediments at mud shrimp sites (Table 2.5). Similarly, Papaspyrou et al. (2005) found higher carbon in the burrow wall for the Mediterranean mud shrimp, *Pestarella tyrrhena,* and suggested a number of possible explanations for why burrow walls are enriched with organic matter, including the adhesion of phytoplankton to burrow linings during irrigation. *U. pugettensis* can remove phytoplankton from the overlying water column (Griffen et al. 2004) and by actively drawing down phytoplankton or suspended material into the sediments, the shrimp facilitate storage of organic material which they could feed on directly as it degrades (Papaspyrou et al. 2005). This storage of plant material within the sediment is evident through elevated carbon content and phaeopigment concentrations in sediment samples from sites where *U. pugettensis* were sampled (Table 2.5). Sediment samples, including burrow walls, were also rich in 16:0, 16:1ω7 and 20:5ω3 FAs indicating a substantial amount of algal material and diatoms in the top layers of the sediment. While the shrimp themselves were not rich in bacterial FAs, sediment samples collected from mud shrimp sites were rich in odd-chain and 18:1ω7 bacterial markers indicating high levels of microbial activity within the sediments (Parrish 2013; Table S4).

SI and FA analyses showed spatial variation in the diets of *U. pugettensis* across the three sample sites within Yaquina Bay, but less so than those for *N. californiensis*. Diets of shrimp from the downriver locations (IF and RAC) were comprised of a mixture of sources and this was consistent between SI and FA data. Primary contributors were POM, SPOM and epiphyte sources and, similar to *N. californiensis*, FA profiles of *U. pugettensis* suggest diatoms are an important source of nutrition in the lower estuary.

POM and epiphyte samples from mud shrimp areas had high levels of the marine diatom biomarkers, EPA and 16:1/16:0. Sedimentary carbon was more deplete in ^{13}C than it was at ghost shrimp sites suggesting *U. pugettensis* may be feeding on allochthonous material in SPOM with terrestrial or riverine origin (Raymond & Bauer 2001, Zhang et al. 2007). *U. pugettensis* also coexist with eelgrass beds in the lower estuary (Dewitt et al. 2004, Dumbauld & D'Andrea unpublished data) meaning detrital material from seagrass production at these sites is more available and may be incorporated into shrimp diets as occurs with other thalassinidean shrimps (Abed-Navandi & Dworschak 2005).

The UPR location is very close to the river channel and characterized by relatively sparse vegetation (Kentula & DeWitt 2003). Therefore it is not surprising that the SIAR model indicated a lower contribution of eelgrass or *Ulva* to diets at this location. Similar to what was observed for upriver *N. californiensis*, FA profiles for *U. pugettensis* showed upriver diets were dominated by dinoflagellates and vascular plants. POM sampled from UPR was also enriched with ^{13}C (-20.43 ± 0.43‰) suggesting marine sourced carbon present in the water column at this site during the time sampling was done. In contrast, SPOM samples were high in detrital markers and depleted in ^{13}C (~ -25‰) suggesting storage of riverine carbon in upriver sediments. The UPR site is situated far enough up river that it may represent a transitional site where tidal changes have a strong influence on whether the shrimp are exposed to marine POM or freshwater POM. The shrimp themselves were relatively depleted in ^{13}C (-19.65 ± 0.38 ‰) confirming a mixed diet of freshwater and marine carbon sources in diets.

Influence of diet on burrowing shrimp population demographics

FA analyses revealed significant intraspecific and interspecific differences between diets of the two burrowing shrimp species in Yaquina Bay that are correlated with the spatial differences in food sources. SI data indicate that the burrowing shrimp we analyzed were feeding on the same primary producers, but the relative importance of the different diet sources was variable. Contrary to suggestions gleaned from burrow architecture alone, our data suggests that *U. pugettensis* obtain a fair amount of nutrition

from benthic sources whereas *N. californiensis* are more dependent on phytoplankton and algal production. Overall, diets of *N. californiensis* are variable and reflect the abundance of local food sources while diets of *U. pugettensis* are more uniform, but spatial sampling for them was more restricted. *N. californiensis* exhibited the most flexibility in their diet and appeared to be generalist feeders adapted to utilizing food in heterogeneous environments (Kassen 2002; Dennis et al. 2011). By maintaining flexibility in feeding mode, these shrimp are able to sustain dense populations (> 400 individuals m^{-2}) and occur on > 80% of the intertidal flats in Yaquina Bay (DeWitt et al. 2004).

Chemical and physical properties of estuaries are spatially variable and ultimately influence the quality of foods available to consumers. Much work has focused on how nutrition influences somatic growth in crustaceans (Hartnoll 2001, Sui et al. 2007, Martin-Creuzburg et al. 2010, Galloway et al. 2014). Both *N. californiensis* and *U. pugettensis* are sedentary bottom dwellers and thus rely on food sources that are available from *in situ* production or that are transported from outside sources by currents and tides. Variation in the quality of available foods may influence population demographics and lead to spatial patterns in growth and survival. Bosley & Dumbauld (2011) observed differences in average body size among spatially separated populations of *N. californiensis* and hypothesized that food quality could be important in limiting growth rates. Average body size for *N. californiensis* varies within the relatively small Yaquina Bay estuary (Bosley unpublished data). We found significant differences in the FA composition of spatially separated populations of shrimp within Yaquina Bay which supports the hypothesis that food may, in part, influence demographic patterns.

There are a number of essential FAs (EFA) required by marine organisms for normal growth and development that cannot be synthesized in adequate amounts "de novo" from shorter chain dietary precursors (Martin-Creuzburg et al. 2010, Parrish 2013). These include the highly unsaturated FAs (HUFA); EPA, DHA, and arachidonic acid (ARA, 20:4ω6). Previous studies showed significant increases in growth, survival and reproductive output with addition of EPA and DHA to crustacean diets (D´Abramo 1989, Becker & Boersma 2007, Sui et al. 2007, Galloway et al. 2014). *N. californiensis* in the

lower estuary (SWI) had the best condition as determined by total FA content and percent organic material per shrimp wet weight. Shrimp from this location also exhibit higher growth rates and reproduce earlier in the season than upriver populations (Bosley & Dumbauld, 2011, Bosley unpublished data). This increased shrimp condition and growth may result from a diet rich in EPA and other marine based HUFA.

The primary nutritional source for *N. californiensis* at SWI was POM followed by a mixture of several sources including epiphytes. Using stable isotope ratios, Shimoda et al. (2007) found that several species of algae including *Sargassum spp*. contributed to the diet of *Nihonotrypaea petalura,* a shrimp inhabiting areas near boulder fields where these algae attached in Japan. Visual inspection of the epiphytes from eelgrass blades at the lower river locations in Yaquina Bay revealed ample amounts of the small red alga, *Smithoria naiadum* (Gayle Hansen, personal communication) which may explain the elevated level of red algae biomarker in shrimp from SWI. Red algae are high quality food sources for marine invertebrates (Galloway et al. 2014, Schmid et al. 2014) and contain high levels of the EFAs, in particular EPA and ARA (Galloway et al. 2012). Further, elevated growth rates occurred in marine isopods fed a diet of the epiphytic red algae, *Smithora naiadum* (Galloway 2014). During the time of year that our study took place, *S. naiadum* likely provides a high quality food source for *N. californiensis* and contributed to elevated condition and possibly growth in the lower estuary.

Interestingly, we also found *N. californiensis* from the most upriver location (TI) to have good condition. This is most likely because of diets rich in DHA along with labile carbon and nitrogen as suggested by the lower C:N ratio (POM; 5.61 ± 0.06) of the primary food sources there. Idaho flats shrimp were lowest in condition and smallest in size. Shrimp at this location are also very abundant (>400 individuals m^{-2}). The negative relationship between shrimp condition and macroalgae biomarkers suggests decomposed macroalgae is a poor diet for *N. californiensis*. *Ulva* collected from Yaquina Bay did not contain DHA or EPA in detectable levels but did have low levels of alpha linolenic acid (ALA, 18:3ω3), and linoleic acid (LIN, 18:2ω6). Other studies have shown EPA and DHA to promote better growth in penaid shrimp than ALA or LIN likely because shrimp

have a limited ability to bioconvert C_{18} PUFAs to longer chain EFAs (Merican & Shim 1996, Lim et al. 1997). Density-dependent competition, food limitation, and availability of lower quality foods like *Ulva* may explain why *N. californiensis* at IF tend to have low condition. *N. californiensis* are strong bioturbators capable of reworking sediments quickly to increase food availability (Fritz 2002). Sediments at IF had the lowest % organic matter with relatively high Chl *a* concentrations suggesting quick reworking of the sediment and consumption of surface algal material which may provide additional EFA to their diets (Table 2.5).

Condition of *U. pugettensis* was statistically uniform as determined by total FA content and percent organics. Differences among spatially separated populations of *U. pugettensis* appear to be subtler than *N. californiensis* and this may result from homogeneity in mud shrimp habitat. *U. pugettensis* typically inhabit lower intertidal areas (-0.5 to +0.5 m, D'Andrea et al unpublished) that are close to tidal channels and do not extend into the oligohaline reaches of the estuary (DeWitt et al. 2004, D'Andrea & Dewitt 2009). As a result, *U. pugettensis* may not be subject to the extreme variability in food quality affecting *N. californiensis* populations. Through the range we sampled *U. pugettensis* was relatively uniform in their percentage FA and biomarker composition, however, shrimp from the upriver locations appear to assimilate a greater proportion of DHA, which was likely acquired from POM. The total omega-3 FAs in POM were also fairly uniform throughout their range which may explain the consistency in shrimp condition.

Availability of high quality nutritional sources may influence the reproductive output of burrowing shrimp populations. Several studies have found nutrition to influence reproduction and condition of offspring in small crustaceans including *Daphnia* spp. (Gliwicz & Guisande 1992, Brett 1993, Becker & Boersma 2007) and the calanoid copepods *Acartia tonsa, Acartia hundsonica* (Jonasdottir 1994), and *Calanus helgolandicus (*Lacoste et al. 2001). For example, female *Daphnia* cultured on high-quality algae produced offspring of higher quality, with positive effects on offspring population growth and life history (Brett 1993). While *N. californiensis* may be capable

of surviving for many years in low quality environments, the quality and quantity of larvae produced by these populations may suffer (Bosley unpublished). Dumbauld et al. (1996) showed fecundity increased with body size, but this relationship was highly variable.

While not explicitly tested, we suggest that shrimp populations in habitats with an abundance of high quality foods allocate greater resources to growth and reproduction which may be important in producing viable offspring. To investigate this hypothesis future work should consider food quality and maternal effects on growth, development and survival of burrowing shrimp larvae. Dedicated feeding studies will reveal underlying mechanisms through which nutrition influences burrowing shrimp development and survival, provide information that will contribute to predicting future climate impacts on estuarine ecosystem structure and function along the west coast of North America.

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Figure 2.1 Sample locations where *N. californiensis*, *U. pugettensis*, and food sources were collected in Yaquina Bay, Oregon, USA. SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island
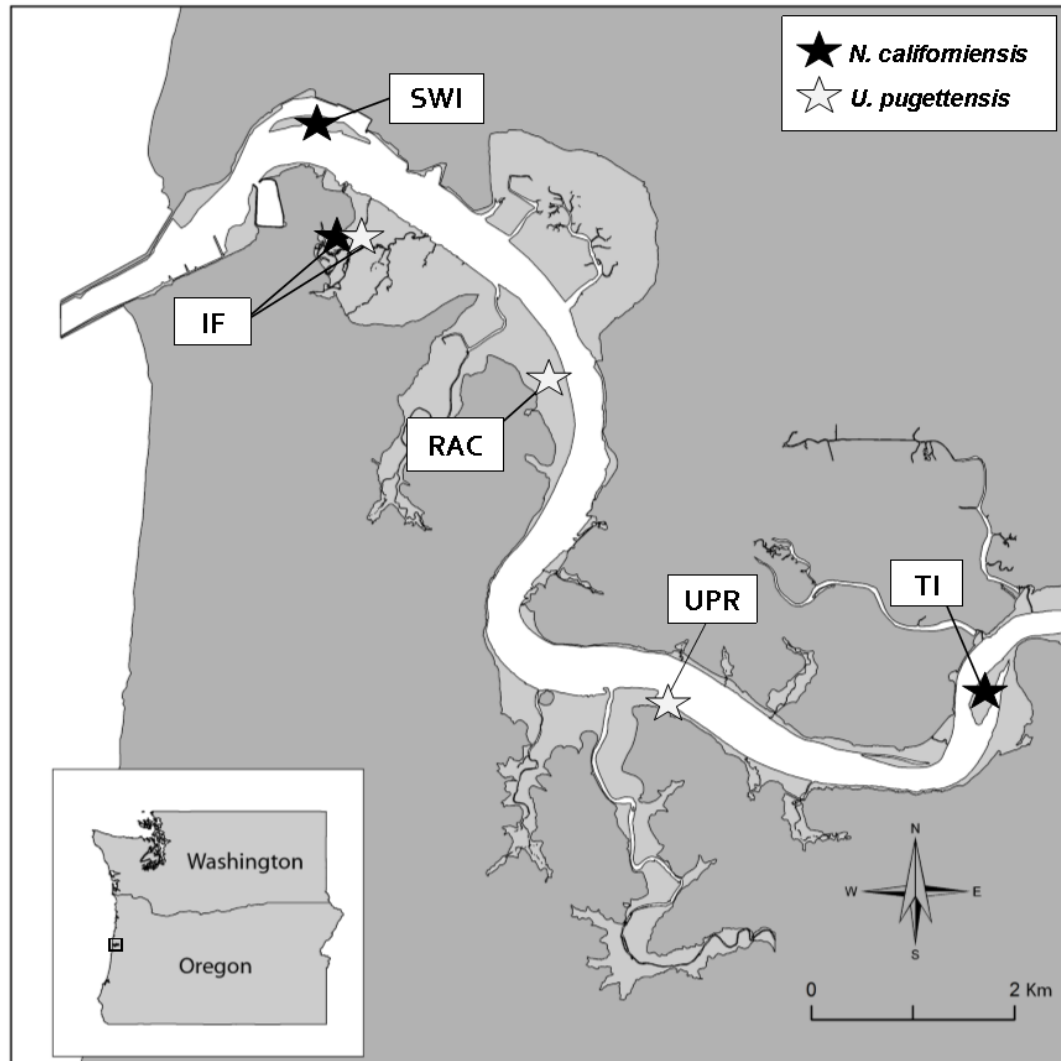
Figure 2.2 Boxplots of $\delta^{13}C$ (a) and $\delta^{15}N$ (b) and for burrowing shrimps collected in Yaquina Bay, Oregon, USA. Boxes are ordered left to right corresponding to increasing distance from mouth of the estuary. Box colors denote the two species, *N. californiensis* and *U. pugettensis*. Significant differences in means ($p < 0.05$) are denoted by different letters (one-way ANOVA and Tukey's pairwise comparison test). SWI; Seawall Island, IF$_N$; Idaho Flats (*N. californiensis* location), IF$_U$; Idaho Flats (*N. pugettensis* location), RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island
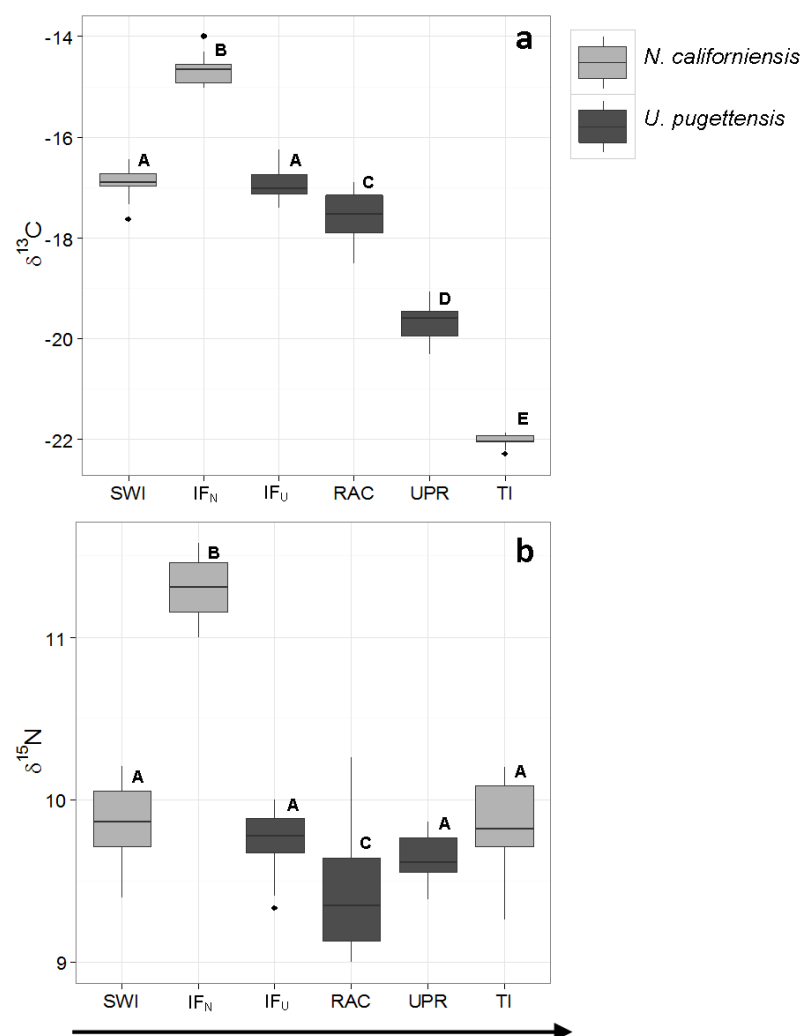
Figure 2.3 Diet compositions for *N. californiensis* and *U. pugettensis*, as estimated by Stable Isotope Analysis in R (SIAR) using 5 sources (with 50%, 75% and 95% credibility interval). Site locations are ordered left to right corresponding to increasing distance from estuary mouth. SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island. See Table A2 in the appendix for SIAR output values
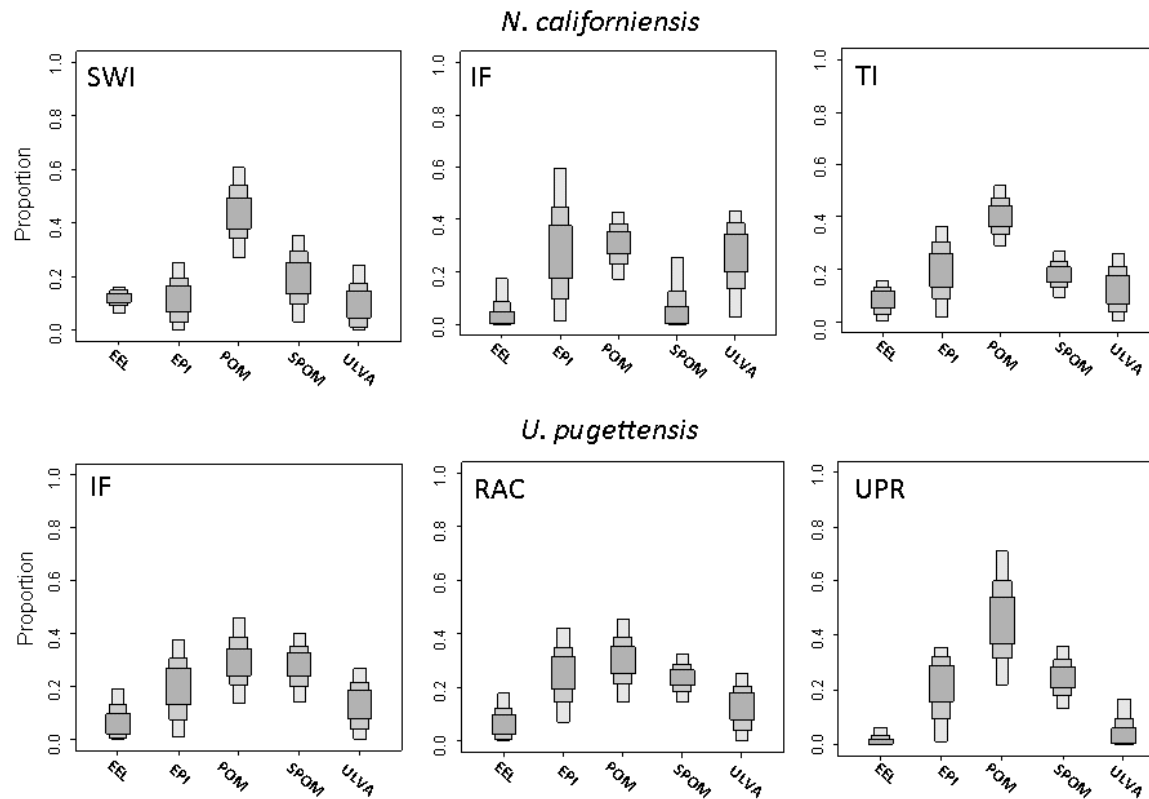
Figure 2.4 Dendrogram showing mean dissimilarities from multi-response permutation procedure (MRPP) of burrowing shrimp fatty acid (FA) compositions by site. *U. pugettensis* sites are shown in grey and *N. californiensis* sites are shown in black. Boxes represent dissimilarity values of branch lengths and arrows point in the direction of increasing distance from estuary mouth. SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island
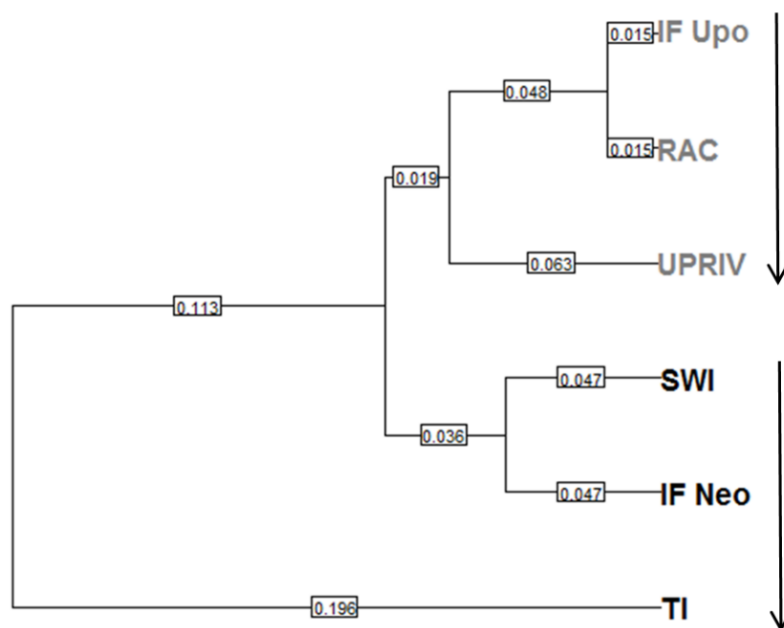
Figure 2.5 a) Non-metric Multidimensional scaling (NMDS) ordination plots of FA compositions for *N. californiensis* b) NMDS plot of *U. pugettensis* FA compositions. Plots show with overlay of locations and biomarker vectors correlations. Only biomarker correlations with $r^2 > 0.8$ are shown. Shaded area represents area species clusters in fatty acid space. See Table 4 for FAs used to calculate biomarkers. Bac; Bacterial, CP; Chlorophyte, D1; Diatom1, D2;Diatom2, Din1;Dinoflagelate1, Din2;Dinoflagellate2, MA; Macroalgae, RP; Rhodophyte, VP; vascular plants, TFA; total fatty acids. SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island
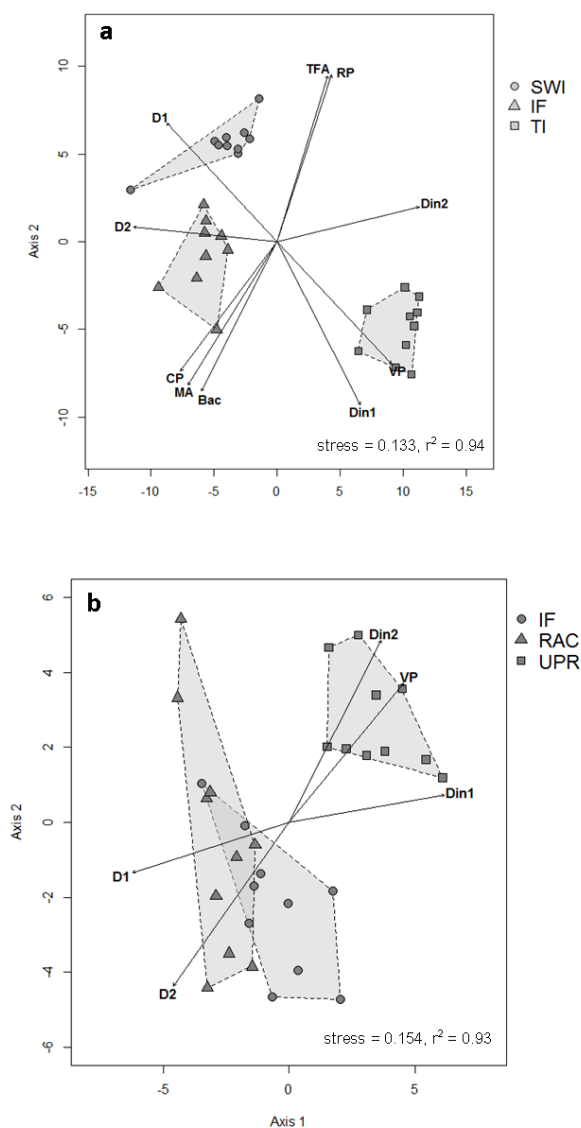
Figure 2.6 Boxplots showing total FA µg mg^{-1} wwt (a) and percent organics (b) from shrimp tissue samples. Boxes are ordered left to right corresponding to increasing distance from mouth of the estuary. Box colors denote the two species, *N. californiensis* and *U. pugettensis*. Significant differences in means ($p < 0.05$) are denoted by different letters (one-way ANOVA and Tukey's pairwise comparison test). SWI; Seawall Island, IF$_N$; Idaho Flats (*N. californiensis* location), IF$_U$; Idaho Flats (*U. pugettensis* location), RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island
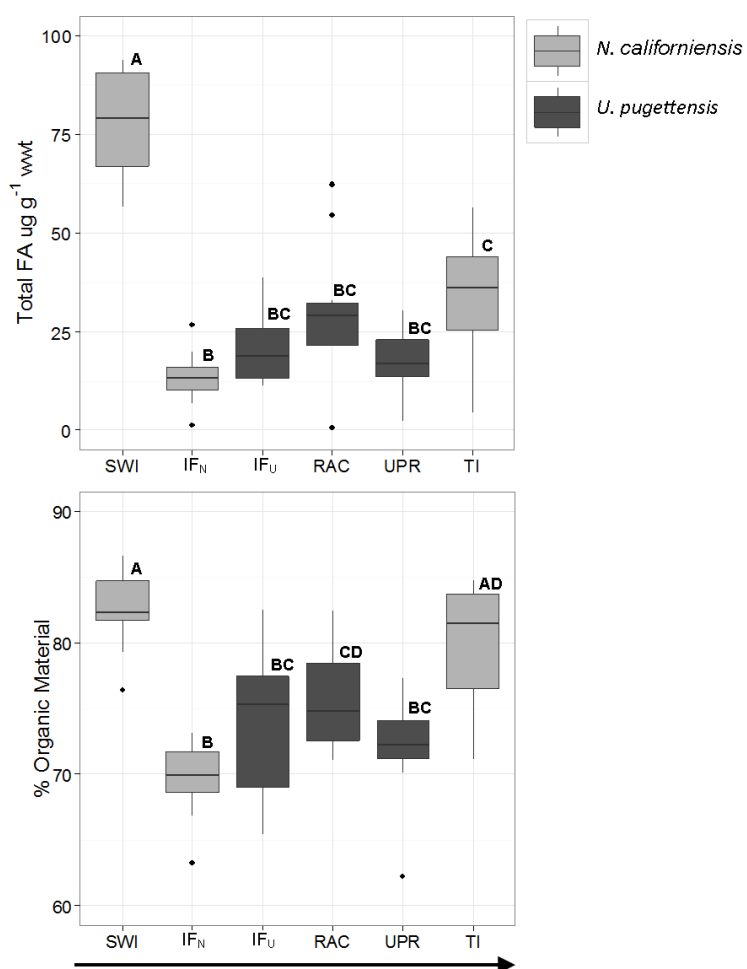
Figure 2.7 Scatterplots showing relationship of percent organic material against FA biomarkers for *N. californiensis* (circles; figs a,b and c) and *U. pugettensis* (squares; fig d,e and f. Biomarker composition shown in table 4. SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island. adj r^2 shown only for significant relationships
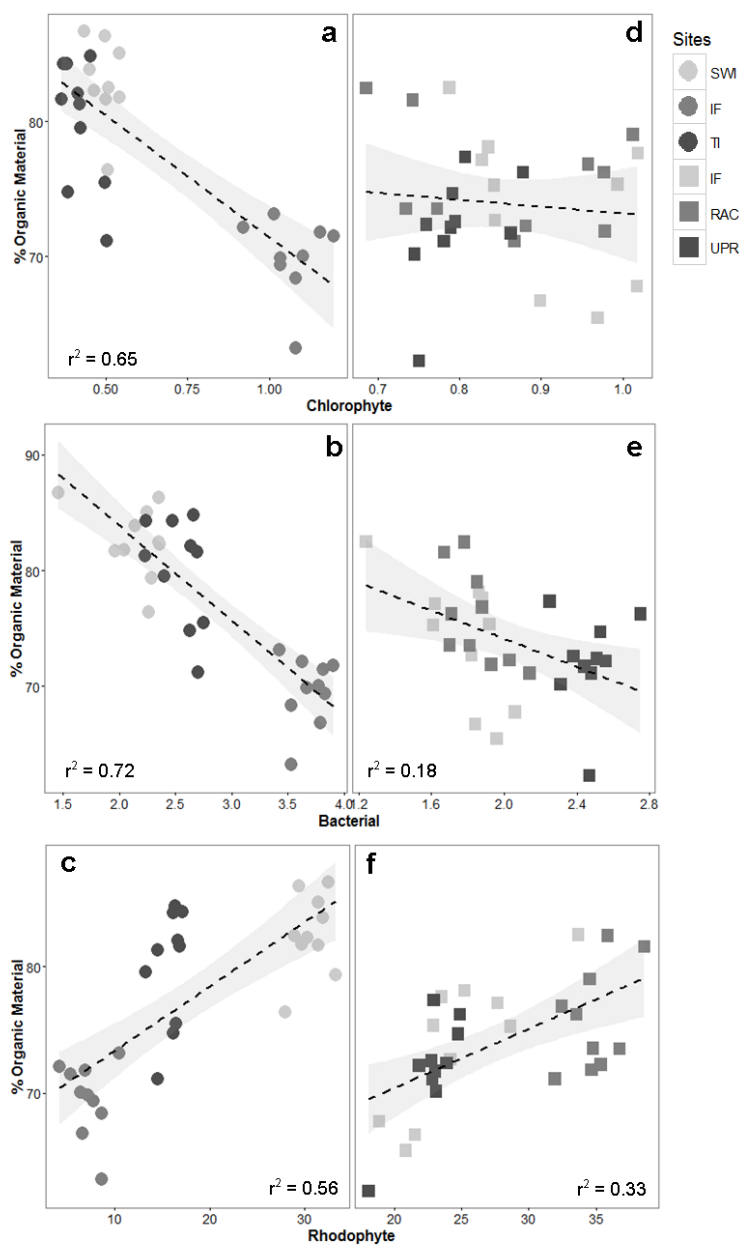
Table 2.1 Summary table of isotope results from tissues of *N. californiensis* and *U. pugettensis*. Significant differences in means ($p < 0.05$) are denoted by different letters (one-way ANOVA and Tukey's pairwise comparison test). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island

| | *N. californiensis* | | | | | | *U. pugettensis* | | | | | |
| | SWI (13) | | IF (11) | | TI (11) | | IF (12) | | RAC (12) | | UPR (12) | |
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
delC	-16.89	0.32	-14.66	0.31	-22.02	0.13	-16.92	0.35	-17.58	0.55	-19.65	0.38
delN	9.88	0.23	11.30	0.21	9.85	0.29	9.75	0.21	9.44	0.37	9.64	0.15
% C	43.99	1.27	40.56	1.14	40.31	1.13	40.46	1.07	41.44	1.59	40.46	0.84
% N	10.33	0.58	10.29	0.45	8.59	0.47	9.49	0.89	10.45	1.07	9.98	0.87
C:N	4.27	0.25	3.94	0.13	4.70	0.31	4.30	0.42	3.99	0.30	4.08	0.35

Table 2.2 Summary table for primary FAs (> 1% total identified fatty acids) and stable isotopes in suspended particulate organic matter (POM). SFA, saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; ω3, omega-three fatty acids, docosahexaenoic acid (DHA, 22:6ω 3), eicosapentaenoic acid (EPA, 20:5ω3). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island.

	PARTICULATE ORGANIC MATERIAL									
	SWI (2)		IF (2)		RAC (2)		UPR (2)		TI (2)	
FAME	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	13.14	0.22	11.91	0.32	12.86	0.07	12.65	0.44	4.02	0.23
ai 16:0	1.02	0.01	0.68	0.33	0.82	0.08	1.02	0.03	0.18	0.02
16:0	15.32	0.33	16.64	0.27	15.66	0.99	14.10	0.20	19.15	0.23
16:1ω11	2.49	0.05	2.25	0.12	1.92	0.42	2.55	0.05	0.39	0.04
16:1ω7	14.52	0.04	12.92	0.08	13.27	0.39	13.22	0.51	3.39	0.25
i 17:0	2.03	0.05	1.35	0.02	1.82	0.10	2.05	0.09	0.75	0.04
16:2ω4	2.44	0.01	2.24	0.02	2.18	0.04	2.52	0.00	0.76	0.04
16:3ω4	1.24	0.01	1.59	0.01	1.18	0.02	1.35	0.00	0.48	0.17
16:4ω1	4.33	0.07	4.10	0.10	4.54	0.04	5.73	0.32	1.11	0.12
18:0	1.05	0.06	2.23	0.18	0.97	0.17	0.75	0.07	3.13	0.01
18:1ω9	2.72	0.20	3.04	0.00	1.49	0.11	1.23	0.04	1.87	0.03
18:1ω7	1.08	0.01	2.04	0.17	0.91	0.18	0.68	0.06	2.36	0.06
18:2ω6	1.34	0.01	1.33	0.03	1.30	0.00	1.23	0.00	2.19	0.01
18:3ω3	0.53	0.01	0.45	0.03	0.70	0.02	0.63	0.06	4.05	0.21
18:4ω3	3.95	0.12	2.47	0.03	4.68	0.07	4.47	0.00	7.00	0.34
20:1ω11	0.42	0.00	0.00	0.00	0.50	0.01	0.41	0.01	0.70	0.39
20:4ω6	0.44	0.00	1.03	0.04	0.41	0.05	0.39	0.01	0.43	0.02
20:5ω3	21.05	0.07	20.57	0.06	22.86	1.13	25.35	0.44	21.21	0.31
22:5ω3	0.28	0.01	0.65	0.01	0.32	0.02	0.26	0.04	1.02	0.01
22:6ω3	6.68	0.44	7.16	0.47	7.79	0.30	6.33	0.33	19.53	0.14
Σ SFA	30.12	0.60	31.78	0.39	29.97	1.22	27.93	0.17	27.02	0.12
Σ MUFA	21.96	0.19	21.17	0.03	18.65	0.20	18.59	0.36	9.39	0.17
Σ PUFA	15.39	0.39	14.53	0.03	16.29	0.19	17.32	0.16	19.08	0.07
Σ ω3	26.23	0.28	24.16	0.06	29.04	1.33	31.15	0.50	33.76	0.08
DHA/EPA	0.32	0.02	0.35	0.02	0.34	0.00	0.25	0.01	0.92	0.02
	SWI (2)		IF (2)		RAC (2)		UPR (2)		TI (2)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
delC	-19.26	0.21	-21.00	0.10	-20.67	1.26	-20.43	0.05	-25.50	0.35
delN	7.86	0.13	7.66	0.03	7.61	0.40	7.12	0.01	9.30	0.06
% C	13.52	0.48	10.74	0.19	10.91	0.28	10.38	0.09	21.87	0.20
% N	2.11	0.06	1.63	0.06	1.49	0.19	1.40	0.00	3.91	0.01
C:N	6.4	0.06	6.61	0.14	7.39	0.78	7.41	0.06	5.61	0.06

[a]**ΣSFA**; 14:0,15:0,16:0, 17:0,18:0, 19:0, 20:0,22:0, 23:0, 24:0; [b] **ΣMUFA**; 14:1, 15:1, 16:1ω11, 16:1ω9, 16:1ω7,16:1ω5, 17:1, 18:1ω11, 18:1ω7, 18:1ω6,18:1ω5, 20:1ω11, 20:1ω9, 20:1ω7, 22:1ω11(13), 22:1ω9, 22:1ω7, 24:1; [c] **ΣPUFA**; 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2, 18:2ω4, 18:3ω6, 18:3ω4, 18:3ω3, 18:4ω3, 18:4ω1, 18:5ω3, 20:2, 20:2ω6, 20:3ω6,20:4ω6, 20:3ω3, 20:4ω3, 20:5ω3, 22:2NIMD,21:5ω3, 22:4ω6, 22:5ω6, 22:4ω3, 22:5ω3, 22:6ω3

Table 2.3 Summary table for primary FAs (> 1% total identified fatty acids) and stable isotopes in epiphytes and the eelgrass, Z*ostera marina*. SFA, saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; ω3, omega-three fatty acids; docosahexaenoic acid (DHA, 22:6ω 3), eicosapentaenoic acid (EPA, 20:5ω3). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island

| | *Z. marina* | | | | | | | | EPIPHYTES | | | | | | | |
| | SWI (2) | | RAC (2) | | UPR (2) | | TI (2) | | SWI (2) | | RAC (2) | | UPR (2) | | TI (2) | |
FAME	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	2.30	2.03	2.85	3.45	1.42	1.00	0.68	0.02	6.75	2.68	9.56	1.25	9.02	0.14	9.46	0.38
i 15:0	0.54	0.12	0.45	0.16	0.68	0.07	1.19	0.43	0.56	0.03	0.48	0.06	0.59	0.03	0.71	0.10
i 16:0	2.03	0.36	1.71	0.40	2.88	0.22	3.19	4.51	1.58	0.10	0.00	0.00	0.84	1.19	1.65	0.17
ai 16:0	0.00	0.00	0.00	0.00	0.00	0.00	1.63	2.30	0.00	0.00	1.34	0.19	0.79	1.12	0.00	0.00
16:0	29.37	6.17	28.09	5.83	22.58	0.18	32.80	4.18	17.16	4.68	11.27	0.42	12.17	0.50	11.60	0.01
16:1ω11	3.73	0.73	3.22	1.01	5.56	0.54	9.30	5.14	3.64	0.70	2.64	0.91	3.28	0.76	4.05	0.53
16:1ω7	5.80	5.79	8.45	10.76	2.93	2.09	0.92	0.24	10.37	3.30	15.96	1.25	14.47	0.69	12.24	0.38
i 17:0	2.63	1.16	2.23	0.61	1.83	0.41	3.02	0.45	1.19	0.47	0.93	0.02	1.19	0.08	1.66	0.06
16:2ω4	0.75	0.65	0.81	0.94	0.54	0.37	0.16	0.05	3.14	1.37	3.72	0.08	4.42	0.30	4.94	0.29
16:3ω4	0.40	0.33	0.43	0.61	0.92	0.58	0.23	0.00	2.35	1.09	4.14	0.64	5.86	0.26	11.16	0.01
17:1	1.01	0.05	1.68	1.03	3.60	1.17	1.49	0.14	0.05	0.07	0.04	0.06	0.00	0.00	0.00	0.00
16:4ω1	1.19	0.97	1.28	1.57	0.70	0.49	0.05	0.07	4.94	2.17	6.46	0.03	5.68	0.29	3.72	0.40
18:0	3.49	0.12	2.73	0.17	2.43	0.74	3.03	0.40	1.25	0.40	1.10	0.12	1.32	0.24	1.53	0.69
18:1ω9	1.71	0.10	1.34	0.13	0.82	0.05	1.29	0.50	0.98	0.14	0.69	0.15	0.59	0.17	0.67	0.02
18:1ω7	1.36	0.19	0.88	0.27	0.78	0.01	0.82	0.07	4.25	2.72	1.86	0.21	1.40	0.14	1.19	0.03
18:2ω6	7.69	5.97	8.17	6.04	7.14	0.59	10.07	1.72	1.24	0.38	0.80	0.09	0.81	0.04	0.56	0.01
18:3ω3	19.36	4.64	20.86	14.23	34.42	8.17	22.98	0.43	0.88	0.05	0.57	0.33	0.59	0.17	0.65	0.07
18:4ω3	0.55	0.78	0.56	0.80	0.24	0.33	0.00	0.00	2.57	0.75	3.47	0.05	3.53	0.46	1.38	0.07
20:0	2.30	0.25	1.64	0.11	1.36	0.15	1.31	0.18	0.15	0.21	0.20	0.02	0.09	0.13	0.00	0.00
20:4ω6	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.07	0.03	0.99	0.21	1.24	0.06	1.32	0.03
20:4ω3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.85	0.43	0.15	0.22	0.00	0.00	0.00	0.00
20:5ω3	5.83	4.10	5.42	7.67	3.91	2.85	0.00	0.00	29.23	5.50	25.76	0.52	25.09	0.50	26.73	0.53
22:0	3.72	0.81	3.04	0.60	2.40	0.07	2.90	0.46	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
22:1ω9	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.65	0.18	0.55	0.09	0.98	0.43	0.00	0.00
22:5ω3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.42	0.59	0.34	0.48	0.20	0.29	0.00	0.00
22:6ω3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	2.80	1.32	3.53	0.45	3.73	0.15	3.62	0.29
Σ SFA	45.41	4.26	42.34	3.61	32.95	2.13	43.49	5.27	25.92	1.18	23.36	1.45	23.08	0.52	23.07	1.09
Σ MUFA	12.60	6.44	13.89	11.91	10.08	2.69	12.33	4.46	20.40	0.28	22.75	1.46	21.30	0.72	18.39	0.24
Σ PUFA	33.66	8.68	35.15	16.94	46.35	6.92	36.39	2.59	18.33	5.22	21.32	1.92	23.46	1.02	23.77	0.84
Σ ω3	25.74	0.24	26.85	5.76	38.56	4.99	22.98	0.43	33.74	4.96	30.07	1.08	29.35	1.15	28.80	0.72
DHA/EPA	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.10	0.06	0.14	0.01	0.15	0.00	0.14	0.01
	SWI (3)		RAC (4)		UPR (2)		TI (4)		SWI (3)		RAC (3)		UPR (3)		TI (3)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
delC	-8.06	0.56	-9.83	0.34	-9.63	0.76	-13.18	0.26	-19.03	1.74	-14.71	0.29	-16.46	1.06	-21.71	0.67
delN	7.00	0.55	6.57	0.55	7.04	0.47	6.46	0.18	6.51	0.44	7.98	0.22	8.11	0.51	7.19	0.22
% C	38.55	3.69	39.70	2.36	41.35	2.53	40.26	1.21	22.02	6.68	11.88	0.82	8.37	0.20	11.35	0.80
% N	3.46	0.76	3.46	0.42	3.72	1.08	4.00	0.23	3.98	0.93	2.13	0.20	1.97	0.22	1.03	0.02
C:N	11.33	1.31	11.58	0.91	10.10	0.86	11.52	2.67	5.47	0.39	5.57	0.20	5.79	0.27	8.13	0.34

Table 2.4 Summary table for primary FAs (> 1% total identified fatty acids) and stable isotopes in *Ulva*. SFA, saturated fatty acids; MUFA monounsaturated fatty acids; PUFA polyunsaturated fatty acids; ω3, omega-three fatty acids; docosahexaenoic acid (DHA, 22:6ω 3), eicosapentaenoic acid (EPA, 20:5ω3). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island.

| | ULVA SP. | | | | | | | | | |
| | SWI (2) | | IF (na) | | RAC (2) | | UPR (2) | | TI (2) | |
FAME	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	0.98	0.09			1.73	0.58	1.20	0.49	1.31	0.06
i 15:0	1.59	0.14			0.75	0.14	1.02	0.02	0.70	0.06
15:0	0.54	0.32			0.49	0.13	0.66	0.39	0.95	0.10
i 16:0	0.00	0.00			1.29	0.48	2.84	0.78	0.61	0.86
ai 16:0	3.11	0.42			0.00	0.00	0.00	0.00	0.76	1.08
16:0	41.04	6.92			52.87	5.82	51.92	1.28	53.97	3.37
16:1ω11	7.89	0.71			2.57	1.39	5.55	0.08	2.52	0.30
16:1ω7	0.87	0.25			1.85	1.13	1.94	1.17	1.49	0.00
i 17:0	4.33	1.25			4.64	0.78	6.94	1.43	3.49	0.09
17:1	1.48	0.67			0.23	0.32	0.67	0.43	0.93	0.41
16:4ω3	5.82	2.31			0.84	0.17	1.05	0.53	2.61	0.36
18:0	1.07	0.73			10.87	6.51	3.32	1.75	1.92	0.69
18:1ω9	1.46	0.28			1.26	0.19	1.03	0.18	1.24	0.13
18:1ω7	15.23	3.55			10.90	2.25	13.01	2.19	12.47	0.86
18:2ω6	2.46	1.23			2.42	0.06	1.92	0.40	5.55	0.72
18:3ω3	7.10	6.25			3.77	0.11	3.42	0.47	7.81	2.31
18:4ω3	2.12	1.55			2.47	0.27	2.26	0.10	1.20	0.46
20:5ω3	0.75	1.06			0.00	0.00	0.00	0.00	0.00	0.00
22:5ω3	0.79	1.11			0.00	0.00	0.00	0.00	0.00	0.00
Σ SFA	43.68	7.99			65.97	1.41	57.10	1.36	58.15	2.84
Σ MUFA	25.47	4.75			16.57	2.33	21.53	1.12	17.72	0.69
Σ PUFA	18.52	12.79			9.89	1.07	9.05	0.75	17.30	2.95
Σ ω3	15.78	11.17			7.07	0.56	6.74	0.90	11.61	2.41
DHA/EPA	0.00	0.00			0.00	0.00	0.00	0.00	0.00	0.00
	SWI (3)		IF (3)		RAC (3)		UPR (3)		TI (3)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
delC	-15.70	1.51	-10.69	0.66	-12.76	2.59	-16.23	1.93	-18.86	0.77
delN	6.39	0.80	8.16	0.04	7.65	0.14	6.97	0.44	6.76	0.54
% C	37.00	1.23	36.35	1.03	35.47	3.61	37.84	2.19	33.22	0.27
% N	5.19	0.11	3.39	0.38	4.27	0.78	4.40	0.47	3.22	0.68
C:N	7.13	0.10	10.77	0.88	8.41	0.92	8.65	0.78	10.60	1.98

[a]**ΣSFA**; 14:0,15:0,16:0, 17:0,18:0, 19:0, 20:0,22:0, 23:0, 24:0; [b] **ΣMUFA**; 14:1, 15:1, 16:1ω11, 16:1ω9, 16:1ω7,16:1ω5, 17:1, 18:1ω11, 18:1ω7, 18:1ω6,18:1ω5, 20:1ω11, 20:1ω9, 20:1ω7, 22:1ω11(13), 22:1ω9, 22:1ω7, 24:1; [c] **ΣPUFA**; 16:2ω4, 16:3ω4, 16:4ω3, 16:4ω1, 18:2, 18:2ω4, 18:3ω6, 18:3ω4, 18:3ω3, 18:4ω3, 18:4ω1, 18:5ω3, 20:2, 20:2ω6, 20:3ω6,20:4ω6, 20:3ω3, 20:4ω3, 20:5ω3, 22:2NIMD,21:5ω3, 22:4ω6, 22:5ω6, 22:4ω3, 22:5ω3, 22:6ω3

Table 2.5 Summary table for primary FAs (> 1% total identified fatty acids) and stable isotopes in sediment particulate organic matter (SPOM) from mud shrimp (*U. pugettensis*) sites. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-three FAs (ω3), docosahexaenoic acid (DHA, 22:6ω 3), eicosapentaenoic acid (EPA, 20:5ω3). Idaho Flats (IF), Raccoon Flats (RAC), Upriver (UPR)

	IF						RAC						UPRIV					
	scrape (2)		core(2)		lining(2)		scrape (2)		core (2)		lining (2)		scrape (2)		core (2)		lining (2)	
FAME	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	4.54	0.08	3.31	0.10	4.01	0.02	4.41	0.54	3.65	0.21	4.01	0.37	4.44	0.08	3.66	0.09	3.80	0.02
i 15:0	2.50	0.57	3.31	0.01	4.18	0.35	2.36	1.22	3.16	0.38	3.56	0.07	3.13	0.40	3.99	0.32	3.90	0.19
ai 15:0	3.42	0.45	5.19	0.25	5.98	0.51	3.67	1.83	5.06	0.27	5.47	0.40	4.41	0.78	6.78	0.82	5.43	0.44
15:0	1.90	0.59	2.68	0.99	2.10	0.00	3.79	2.96	2.66	0.18	1.83	0.01	1.28	0.02	1.86	0.01	1.17	0.06
15:1	0.82	0.15	1.30	0.08	1.43	0.21	0.85	0.33	1.16	0.15	1.11	0.04	1.01	0.15	1.50	0.11	1.10	0.04
i 16:0	0.91	0.25	0.93	0.11	1.17	0.22	0.00	0.00	0.00	0.00	0.00	0.00	0.41	0.58	1.02	0.26	1.15	0.05
ai 16:0	0.00	0.00	0.00	0.00	0.00	0.00	0.85	0.24	1.28	0.34	1.13	0.01	0.50	0.70	0.00	0.00	0.00	0.00
16:0	17.16	0.42	14.56	0.34	15.28	1.35	16.65	1.03	14.94	0.67	13.64	0.40	16.22	0.19	14.18	0.99	13.61	0.08
16:1ω11	2.30	0.83	1.96	0.58	2.48	0.87	1.75	0.43	2.65	0.67	2.69	0.04	2.01	0.32	1.93	0.74	2.82	0.04
16:1ω7	20.20	2.55	19.27	1.07	21.83	3.31	14.82	0.26	14.37	2.19	16.76	0.05	15.50	0.72	15.00	0.54	14.79	0.60
16:1ω5	1.16	0.31	1.56	0.03	2.42	0.18	1.24	0.48	1.69	0.09	2.04	0.09	1.47	0.13	2.28	0.33	2.06	0.17
i 17:0	1.24	0.06	1.08	0.02	1.16	0.11	1.18	0.19	1.09	0.30	0.92	0.01	1.25	0.01	1.24	0.07	1.01	0.02
ai 17:0	0.92	0.11	1.27	0.02	1.31	0.05	0.70	0.33	1.32	0.43	1.08	0.03	1.12	0.08	1.42	0.14	1.10	0.07
16:2ω4	2.08	0.54	1.16	0.03	1.22	0.05	1.61	0.79	1.02	0.18	1.00	0.07	1.72	0.41	0.79	0.02	0.89	0.01
phytanic	0.85	0.21	1.27	0.23	1.10	0.06	0.38	0.53	0.91	0.05	0.89	0.07	0.81	0.06	0.93	0.11	0.68	0.00
16:3ω4	1.42	0.60	3.07	1.33	1.62	0.00	1.74	0.75	1.91	1.08	1.72	0.20	0.86	0.04	1.16	0.10	0.83	0.01
16:4ω3	0.00	0.00	0.10	0.00	0.16	0.01	0.56	0.79	0.77	0.76	0.16	0.08	0.22	0.02	0.22	0.22	0.19	0.04
16:4ω1	1.20	0.22	0.48	0.12	0.40	0.10	1.44	1.12	0.74	0.29	0.65	0.13	0.86	0.21	0.29	0.01	0.40	0.02
18:0	3.49	0.77	3.43	0.01	2.69	0.10	3.08	0.06	3.09	0.29	3.37	0.49	3.39	0.12	4.36	1.94	3.82	0.58
18:1ω9	2.35	0.31	3.21	0.02	2.69	0.19	2.88	0.35	3.56	1.41	3.07	0.27	3.40	0.05	4.16	0.87	3.03	0.29
18:1ω7	9.46	0.63	10.09	0.44	4.78	6.47	7.60	2.72	7.67	0.43	7.84	0.17	7.67	0.16	7.37	0.33	6.75	0.17
18:2ω6	0.94	0.06	0.92	0.05	0.97	0.04	1.15	0.35	1.98	0.33	1.37	0.04	1.32	0.03	1.64	0.15	2.52	1.23
18:3ω3	0.20	0.28	0.46	0.03	0.56	0.04	1.43	1.36	1.94	2.07	0.54	0.07	0.95	0.03	0.90	0.18	0.87	0.02
18:4ω3	0.90	0.14	0.41	0.08	0.33	0.09	1.85	0.01	1.37	1.32	0.34	0.06	0.88	0.18	0.46	0.05	0.53	0.02
20:0	0.34	0.48	0.92	0.08	1.19	0.13	1.16	0.44	1.82	0.40	1.91	0.28	1.71	0.29	3.04	0.17	2.80	0.16
20:1ω11	0.26	0.37	1.52	0.56	0.53	0.09	0.48	0.07	0.93	0.77	0.52	0.35	0.55	0.04	0.72	0.01	0.37	0.02
20:1ω9	0.15	0.22	0.45	0.14	0.17	0.24	0.35	0.00	0.37	0.07	0.42	0.04	0.43	0.00	0.49	0.04	0.41	0.09
20:1ω7	0.35	0.50	0.35	0.01	0.30	0.04	0.94	0.86	0.39	0.01	0.24	0.00	0.43	0.02	0.25	0.05	0.24	0.03
20:2ω6	0.00	0.00	0.17	0.24	0.00	0.00	0.17	0.25	0.91	0.22	0.46	0.65	0.00	0.00	0.00	0.00	0.00	0.00
20:4ω6	1.02	1.45	3.60	0.23	2.86	0.07	0.90	0.04	1.36	0.18	1.61	0.24	1.22	0.14	1.61	0.14	1.50	0.23
20:4ω3	0.00	0.00	0.00	0.00	0.00	0.00	0.18	0.26	0.17	0.24	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
20:5ω3	13.29	1.56	4.92	0.63	3.21	0.82	7.63	5.50	3.43	0.49	3.48	0.57	7.05	1.95	2.16	0.50	2.89	0.01
22:0	0.50	0.71	1.88	0.24	2.61	0.25	2.49	1.16	4.01	0.46	4.34	0.52	2.82	0.33	4.72	0.04	4.79	0.33
22:1ω9	0.82	1.17	0.00	0.00	3.53	1.98	1.83	1.01	0.00	0.00	3.44	0.65	2.18	0.36	0.00	0.00	4.23	1.39
24:0	0.56	0.80	2.24	0.19	2.95	0.15	2.59	1.07	4.46	1.67	5.24	0.78	3.54	0.52	6.09	0.21	5.68	0.38
22:6ω3	1.48	2.09	0.23	0.32	0.00	0.00	2.36	2.15	0.53	0.40	0.27	0.37	2.86	0.65	0.36	0.51	0.94	0.16
Σ SFA	29.35	1.47	30.30	1.30	31.92	1.74	34.54	0.68	35.55	2.48	35.24	2.03	34.20	1.41	38.83	2.55	36.37	0.30
Σ MUFA	38.30	1.91	40.27	1.39	40.89	1.67	33.10	4.25	33.33	1.55	38.72	0.42	35.16	1.21	34.37	1.24	36.51	0.72
Σ PUFA	9.09	2.04	12.25	1.32	14.26	1.95	15.76	1.43	16.60	2.91	15.63	0.18	13.23	0.55	11.79	0.08	16.75	2.08
Σ ω3	14.39	1.42	5.89	0.69	4.26	0.85	11.65	3.61	7.67	3.91	4.52	0.79	9.10	2.14	3.74	0.15	4.47	0.01
DHA/EPA	0.12	0.17	0.04	0.06	0.00	0.00	0.28	0.08	0.15	0.10	0.07	0.10	0.41	0.02	0.14	0.20	0.32	0.06

	IF_UPO						RAC						UPRIV					
	scrape (3)		core (3)		lining (3)		scrape (3)		core (3)		lining (3)		scrape(3)		core(3)		lining(3)	
delC	-21.56	0.20	-22.12	0.11	-22.95	0.06	-24.50	0.41	-25.25	0.19	-25.29	0.06	-25.23	0.05	-25.53	0.08	-25.44	0.07
delN	5.47	0.23	5.48	0.03	5.83	0.06	4.11	0.11	3.69	0.29	3.91	0.04	3.96	0.21	3.73	0.13	3.72	0.06
% C	0.33	0.16	0.48	0.05	2.80	3.01	2.45	0.33	2.17	0.27	2.52	0.34	1.33	0.38	1.66	0.14	1.98	0.19
% N	0.05	0.02	0.06	0.00	0.27	0.30	0.16	0.01	0.13	0.00	0.15	0.02	0.11	0.02	0.12	0.00	0.14	0.01
C:N	7.22	0.86	8.35	0.23	10.31	0.11	15.42	1.27	16.11	1.67	16.60	0.42	11.82	1.16	13.81	0.91	14.05	0.97

Table 2.6 Summary table for primary FAs (> 1% total identified fatty acids) and stable isotopes in sediment samples from ghost shrimp (*N. californiensis)* sites. Saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), omega-three FAs (ω3), docosahexaenoic acid (DHA, 22:6ω 3), eicosapentaenoic acid (EPA, 20:5ω3*)*. SWI; Seawall Island, IF; Idaho Flats, TI; Tongue Island

	SWI				IF_NEO				TI			
	scrape (2)		core (2)		scrape (2)		core (2)		scrape (2)		core (2)	
FAME	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	5.82	0.89	3.78	0.26	2.90	0.12	2.43	0.01	6.05	1.54	3.32	0.79
i 15:0	2.47	0.31	3.43	0.07	0.71	0.02	1.12	0.10	2.33	0.69	4.25	0.09
ai 15:0	2.23	0.33	3.10	0.15	0.45	0.04	0.76	0.04	1.95	0.70	3.93	0.15
15:0	1.49	0.03	1.50	0.11	1.16	0.09	2.49	0.33	1.98	0.18	2.47	1.02
15:1	1.01	0.16	1.30	0.02	0.26	0.01	0.38	0.04	0.74	0.24	1.31	0.00
i 16:0	0.80	0.16	1.08	0.00	0.00	0.00	0.00	0.00	1.00	0.03	1.12	0.28
16:0	18.93	0.38	16.32	0.95	21.60	1.05	20.58	0.26	19.52	0.17	16.56	0.14
16:1ω11	1.46	0.22	2.14	0.13	1.32	0.17	2.06	0.21	2.37	0.40	1.82	0.49
16:1ω7	15.58	0.06	18.59	0.21	41.55	0.51	38.89	1.37	18.33	1.03	16.70	2.46
16:1ω5	1.26	0.03	1.80	0.08	0.43	0.00	0.49	0.01	0.86	0.20	1.48	0.21
i 17:0	1.34	0.17	1.45	0.01	1.01	0.03	0.98	0.07	1.60	0.07	1.56	0.16
ai 17:0	1.03	0.03	1.37	0.03	0.45	0.02	0.53	0.04	0.83	0.56	1.50	0.08
16:2ω4	1.90	0.14	2.11	0.09	1.61	0.06	1.62	0.09	3.16	0.84	1.53	0.38
16:3ω4	1.22	0.77	1.79	1.21	2.93	0.21	2.54	0.22	4.84	0.98	1.59	0.27
17:1	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.05	0.20
16:4ω1	0.96	0.34	0.71	0.10	0.56	0.03	0.40	0.04	1.59	0.71	0.41	0.19
18:0	4.12	0.09	2.81	0.27	1.08	0.09	0.91	0.01	2.85	1.25	7.44	6.84
18:1ω9	6.25	0.50	4.86	0.57	1.94	0.01	2.27	0.00	2.23	0.30	5.16	1.13
18:1ω7	8.46	0.36	10.14	0.28	1.89	0.05	2.23	0.19	3.46	1.07	5.37	0.42
18:2ω6	1.23	0.18	1.20	0.31	0.88	0.04	0.89	0.00	1.03	0.16	2.88	2.72
18:3ω3	1.21	0.31	1.04	0.00	0.17	0.01	0.23	0.02	1.14	0.33	1.65	0.23
18:4ω3	1.82	0.49	0.83	0.07	0.92	0.03	0.89	0.07	1.78	0.41	0.86	0.23
20:0	0.44	0.02	0.33	0.01	0.12	0.00	0.12	0.01	0.49	0.12	1.08	0.23
20:1ω11	2.28	1.15	0.66	0.40	0.08	0.02	0.00	0.00	0.37	0.21	0.15	0.21
20:1ω9	1.11	0.24	0.70	0.04	0.11	0.01	0.07	0.00	0.27	0.07	0.38	0.10
20:1ω7	0.97	0.40	0.62	0.03	0.16	0.03	0.11	0.00	0.29	0.00	0.12	0.17
20:4ω6	1.11	0.08	1.98	0.20	2.97	0.07	5.86	0.20	1.15	0.22	2.55	0.02
20:4ω3	0.00	0.00	0.00	0.00	0.12	0.01	0.06	0.08	0.23	0.32	0.00	0.00
20:5ω3	7.92	1.98	9.25	0.40	8.39	0.56	7.20	0.21	10.68	2.62	4.96	1.75
22:0	0.00	0.00	0.00	0.00	0.26	0.03	0.11	0.16	0.77	0.18	1.23	0.01
22:1ω9	0.00	0.00	0.00	0.00	0.72	0.10	0.00	0.00	0.73	0.24	0.00	0.00
24:0	0.00	0.00	0.00	0.00	0.26	0.01	0.38	0.06	0.97	0.49	1.53	0.08
22:6ω3	3.13	0.71	1.97	0.18	0.53	0.01	0.25	0.02	1.99	0.16	0.58	0.82
Σ SFA	31.38	1.28	25.35	1.00	27.37	0.96	27.37	0.87	33.15	0.63	34.40	5.22
Σ MUFA	39.11	0.61	41.90	0.97	48.49	0.33	46.53	0.94	30.17	2.67	33.58	3.03
Σ PUFA	9.88	0.68	10.06	0.86	11.38	0.46	12.75	0.91	16.80	2.89	12.78	1.39
Σ ω3	11.11	1.29	11.26	0.51	9.59	0.59	8.37	0.38	13.93	2.89	7.54	2.21
DHA/EPA	0.40	0.01	0.21	0.01	0.06	0.00	0.03	0.00	0.19	0.03	0.09	0.13
	SWI				IF_NEO				TI			
	scrape (3)		core (3)		scrape (3)		core (3)		scrape (3)		core (3)	
delC	-19.96	0.14	-19.83	0.33	-15.81	0.34	-16.62	0.27	-24.63	0.54	-25.53	0.17
delN	5.82	0.25	5.73	0.26	6.75	0.12	6.48	0.17	3.45	0.16	3.05	0.08
% C	0.11	0.00	0.11	0.02	0.16	0.02	0.13	0.01	0.48	0.08	0.79	0.26
% N	0.02	0.00	0.02	0.00	0.03	0.00	0.03	0.00	0.05	0.00	0.06	0.01
C:N	5.28	0.15	5.16	0.73	5.79	0.26	5.16	0.20	8.84	1.01	12.50	2.32

Table 2.7 Primary FAs (> 1% total identified FA) in *N. californiensis* and *U. pugettensis*. Saturated fatty acids (SFA); monounsaturated fatty acids (MUFA); polyunsaturated fatty acids (PUFA); omega-three FAs (ω3), docosahexaenoic acid (DHA, 22:6ω 3), eicosapentaenoic acid (EPA, 20:5ω3). Significant differences in means for each sample type ($p < 0.05$) are denoted by different letters (one-way ANOVA and Tukey's pairwise comparison test). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island

| | *N. californiensis* | | | | | | | | | | | | *U. pugettensis* | | | | | | | | | | | |
| | SWI n = 10 | | IF n = 10 | | TI n = 10 | | IF n = 10 | | RAC n = 10 | | UPR n = 10 | |
FAME	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
14:0	4.63	0.38	3.00	0.85	3.56	0.24	6.73	0.67	7.24	0.43	5.71	0.70
14:1	2.71	0.33	2.66	0.40	2.60	0.26	0.24	0.03	0.26	0.03	0.31	0.08
16:0	15.52	0.75	14.99	0.84	17.40	0.87	12.04	0.77	11.90	1.26	13.60	0.64
16:1ω7	13.89	0.75	12.46	1.97	8.78	0.87	10.60	0.71	11.90	1.33	8.71	0.63
16:2ω4	3.73	0.18	3.45	0.60	3.15	0.21	2.84	0.36	3.22	0.26	2.47	0.22
16:3ω4	1.17	0.43	0.85	0.30	2.20	0.52	1.46	0.30	1.74	0.15	1.30	0.45
16:4ω1	2.92	0.59	1.11	0.44	0.79	0.20	4.31	0.85	5.10	0.42	2.80	0.52
18:0	2.09	0.74	3.10	0.53	2.99	0.19	2.94	0.39	2.31	0.30	3.59	0.54
18:1ω9	7.72	2.86	4.59	1.82	8.30	1.25	4.97	0.68	5.19	1.11	5.51	0.45
18:1ω7	4.20	0.21	5.40	0.57	3.45	0.30	4.44	0.33	4.36	0.48	4.37	0.21
18:2ω6	0.91	0.04	1.62	0.18	2.03	0.17	1.28	0.13	1.03	0.09	1.64	0.14
18:3ω3	0.46	0.03	0.90	0.15	2.70	0.25	0.74	0.18	0.74	0.23	1.76	0.29
18:4ω3	2.67	0.20	2.13	0.51	9.99	1.03	3.30	0.37	3.34	0.44	4.01	0.57
20:4ω6 (AA)	0.83	0.05	3.46	0.85	0.80	0.11	1.23	0.26	0.80	0.10	1.11	0.14
20:4ω3	0.61	0.04	0.43	0.17	1.44	0.18	0.44	0.24	0.52	0.03	0.45	0.24
20:5ω3 (EPA)	25.34	1.02	23.63	1.15	12.56	1.12	29.30	1.70	27.81	2.49	25.00	1.10
21:5ω3	0.62	0.06	0.48	0.08	0.57	0.05	0.99	0.09	0.96	0.11	0.83	0.30
22:6ω3 (DHA)	4.52	0.34	6.06	0.73	10.24	0.79	6.29	0.62	5.63	0.73	9.31	0.95
Σ SFA[a]	22.74[a]	1.00	22.88[a]	0.67	24.82[b]	0.96	23.54[a]	0.75	23.00[a]	0.82	24.80[b]	0.77
Σ MUFA[b]	30.45[a]	2.48	28.48[a]	2.44	24.95[b]	1.75	22.44[bc]	1.24	23.94[bc]	2.67	21.52[c]	0.84
Σ PUFA[c]	45.33[a]	1.90	46.24[ab]	2.17	48.78[b]	2.40	53.10[c]	1.70	52.11[c]	3.35	52.36[c]	0.86
Σ ω3	34.72[a]	1.43	34.48[a]	2.08	39.10[b]	2.09	41.46[bc]	1.96	39.60[b]	2.95	42.42[c]	0.88
DHA/EPA	0.18[a]	0.01	0.26[b]	0.03	0.82[c]	0.09	0.21[ab]	0.01	0.20[ab]	0.02	0.37[d]	0.03

Table 2.8 Summary table of shrimp size, total FA per wet weight and mean FA biomarker values for both species from all sites sampled. Significant differences in means for total FA sample type ($p < 0.05$) are denoted by different letters (one-way ANOVA and Tukey's pairwise comparison test). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island.

	N. californiensis						*U. pugettensis*					
	SWI (10)		IF (10)		TI (10)		IF (10)		RAC (10)		UPR (10)	
BIOMARKERS (percent total FA)	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Carapace Length (mm)	10.27	0.9	9.52	0.76	13.77	1.97	23.11	4.51	23.1	5.11	27.14	1.95
Total FA (µg mg-1)	82.21[a]	16.78	13.37[b]	7.00	34.50[c]	15.63	20.39[bc]	8.82	30.34[bc]	17.39	17.63[bc]	7.91
Diatom1 (16:1ω7/16:0) [1]	0.90	0.03	0.83	0.13	0.50	0.05	0.88	0.08	1.00	0.04	0.64	0.06
Diatom2 (16:1ω7+20:5ω3)/(22:6ω3 + 18:1ω9 + 18:4ω3) [2]	2.80	1.03	2.89	0.54	0.75	0.11	2.75	0.17	2.82	0.29	1.79	0.09
Bacteria (Σ odd branched FAs) [2]	2.15	0.28	3.69	0.16	2.54	0.19	1.78	0.24	1.85	0.15	2.47	0.14
Dinoflagellate1 (22:6ω3) [1]	4.52	0.34	6.06	0.73	10.24	0.79	6.29	0.62	5.63	0.73	9.31	0.95
Dinoflagellate2 (18:0 + 18:1ω9 + 18:4ω3) [3]	12.48	2.54	9.82	1.54	21.28	1.51	11.21	0.72	10.83	1.04	13.11	0.47
Vascular Plants (18:2ω6 + 18:3ω3) [4]	0.80	0.04	1.13	0.16	3.07	0.24	0.97	0.17	0.99	0.22	1.96	0.29
Macroalgae (20:4ω6) [3]	0.83	0.05	3.46	0.85	0.80	0.11	1.23	0.26	0.80	0.10	1.11	0.14
Chlorophyte (18:1ω7/18:1ω9) [5]	0.49	0.04	1.07	0.08	0.42	0.05	0.90	0.09	0.86	0.12	0.80	0.04
Rhodophyte (20:5ω3/20:4ω6) [4]	30.71	1.70	7.21	1.78	15.82	1.24	24.73	4.33	34.87	1.97	22.83	1.90

Biomarker references
1) Copeman & Parrish (2003)
2) Antonio & Richoux NB (2014)
3) Kelly & Scheibling (2012)
4) Alfaro et al. (2006)
5) Nelson et al. (2002)

Table 2.9 Table showing of chl *a* (mg g^{-1}), phaeopigment (mg g^{-1}) and % organic content from sediment particulate organic matter (SPOM) collected from shrimp sites. Sites are ordered from left to right corresponding to increasing distance from estuary mouth. Significant differences in means for each sample type ($p < 0.05$) are denoted by different letters (one-way ANOVA and Tukey's pairwise comparison test). SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island.

N. californiensis

Sample Type	SWI n = 2			IF n = 2			TI n = 2		
	CHL A	PHEO	% Organic	CHL A	PHEO	% Organic	CHL A	PHEO	% Organic
Scrape	24.47 ± 0.45[a]	19.25 ± 4.58[a]	1.38 ± 0.03[a]	99.46 ± 6.90[b]	29.70 ± 4.30[ab]	1.28 ± 0.18[a]	70.89 ± 36.62[b]	18.19 ± 3.86[a]	2.99 ± 0.17[b]
Core	24.16 ± 7.97[a]	14.69 ± 2.84[a]	1.21 ± 0.05[a]	85.25 ± 0.68[b]	18.00 ± 0.17[a]	1.03 ± 0.07[a]	24.08 ± 2.83[a]	9.22 ± 0.40[a]	3.29 ± 0.13[b]

U. pugettensis

Sample Type	IF n = 2			RAC n = 2			UPR n = 2		
	CHL A	PHEO	% Organics	CHL A	PHEO	% Organics	CHL A	PHEO	% Organics
Scrape	7.75 ± 10.23[a]	28.38 ± 3.97[ab]	2.02 ± 0.52[ab]	70.28 ± 16.08[b]	52.52 ± 12.49[c]	6.28 ± 0.26[c]	22.94 ± 6.92[a]	37.88 ± 3.50[b]	5.09 ± 0.42[c]
Core	26.59 ± 0.14[a]	25.18 ± 1.58[b]	2.28 ± 0.52[ab]	65.30 ± 16.33[b]	35.52 ± 10.44[c]	7.59 ± 0.34[c]	24.05 ± 14.8[a]	18.99 ± 0.01[b]	5.83 ± 0.18[d]
Lining	16.31 ± 4.69[a]	19.31 ± 3.49[a]	3.46 ± 0.15[a]	23.65 ± 2.45[a]	54.05 ± 31.44[b]	7.35 ± 0.51[b]	25.35 ± 8.54[a]	35.80 ± 2.85[ab]	6.89 ± 0.48[b]

CHAPTER 3

**EVALUATION OF ENVIROMENTAL EFFECTS ON AGE AND GROWTH
IN THE BURROWING SHRIMPS, *UPOGEBIA PUGETTENSIS*
AND *NEOTRYPAEA CALIFORNIENSIS***

ABSTRACT

Crustaceans are ecologically and economically important organisms as they play an
essential role in marine and estuarine ecosystems and their fisheries are highly valuable.
Their importance has lead managers to seek methods to develop population models and
assess stock status which requires knowledge of age and growth. The lipofuscin aging
method has proven to be a viable alternative for aging crustaceans where size does not
correlate with age, but broad scale application requires validation of the method and
confirmation that accumulation of lipofuscin is constant over a range of environmental
conditions. This study used a combination of laboratory mesocosm experiments and field
growth studies to determine how environmental viability influences lipofuscin
accumulation in the burrowing shrimps, *Neotrypaea californiensis* and *Upogebia
pugettensis*. Data from mesocosm temperature trials showed a significant effect of
elevated temperature on lipofuscin accumulation in *N. californiensis* where no
temperature effect was observed for *U. pugettensis*. Field growth studies of *N.
californiensis* showed that change in body size was correlated to both time and location
but lipofuscin accumulation rate was similar across sites. Field experiments for *U.
pugettensis* showed lipofuscin concentration was not related to age, but size was strongly
correlated with temperature-degree days (TDD) suggesting growth patterns could be
predicted by time and thermal history. Results of this study indicate that lipofuscin aging
methods can be widely applied to conduct demographic assessments in wild populations
of *N. californiensis in* Yaquina Bay, Oregon, and potentially other bays whereas size-
based metrics are adequate for estimating age of the mud shrimp, *U. pugettensis*.

INTRODUCTION

Crustaceans are important components of marine and estuarine ecosystems. They play an essential role in food webs (Ackman et al. 1970, Orlov 1998, Croxall et al. 1999) and many have lifestyles that facilitate biogeochemical processes in benthic environments (Webb & Eyre 2004, Kristensen & Alongi 2006, D'Andrea & Dewitt 2009). They are also an important economic resource. In 2013, over 1.8 billion dollars in revenue were acquired through crustacean fisheries landings from US waters (Voorhees 2014). Because of their ecological and economic importance, there is a need to understand crustacean population dynamics and produce stock assessments to inform conservation and management frameworks (Smith & Addison 2003, Buhay 2011). However, development of population dynamics models has been hindered by the difficulty in aging crustaceans and therefore methods for estimating their population parameters have generally focused on length-based metrics (Fogarty & Idoine 1988, Zheng et al. 1995, Hartnoll 2001, Smith & Addison 2003, Punt et al. 2014). These length-based approaches involve complicated statistical models and require knowledge of variability in crustacean growth (Punt 2003).

Crustacean growth patterns have been a prominent research topic for several decades. They exhibit a discontinuous growth process that is characterized by a molting event followed an intermolt period (reviewed in Hartnoll 1982, 2001). Numerous studies have shown temperature, food, and salinity have a significant effect on crustacean growth (Hartnoll 1982, Oh & Hartnoll 2000, Paglianti & Gherardi 2004, Fockedey et al. 2005, Brylawski & Miller 2006, Hufnagl & Temming 2011, Stoner et al. 2013). These primary factors tend to be highly variable in marine and estuarine environments which can lead to a wide range of size-at-age within and among cohorts (Ju et al. 2001, Bosley & Dumbauld 2011). Because of the problems associated with estimating age based on individual size, researchers have begun to explore alternative ageing methods to provide robust estimates of crustacean age, including analysis of the biochemically produced ageing pigment, lipofuscin (Bluhm & Brey 2001, Puckett et al. 2008, Harvey et al. 2010, Bosley & Dumbauld 2011).

Lipofuscin is a an auto-fluorescent pigment that accumulates in animal tissues as a by-product of cellular metabolism (Terman & Brunk 1998, 2004). Found in post-mitotic cells, the pigment increases in concentration as an animal ages (Sheehy et al. 1994, 1995, Brunk & Terman 2002, Terman & Brunk 2004) and has proven to be a useful age biomarker for arthropod species where morphological measurements are weakly correlated to actual age (Ettershank 1983, Sheehy et al. 1998, Ju et al. 1999, Bluhm et al. 2001, Kodama et al. 2006, Maxwell et al. 2007, Bosley & Dumbauld 2011, McGaffin et al. 2011). Lipofuscin is produced through a metabolic process and therefore the rate at which it accumulates is linked to temperature and physiological stress (O'Donovan & Tully 1996, Brunk & Terman 2002). This link to the environment has limited the broad scale application of lipofuscin aging methods when temperature records may not be available (Wahle et al. 1996, Sheehy & Prior 2008). Bosley and Dumbauld (2011), however found no difference in lipofuscin accumulation rate across spatially separated populations of the burrowing ghost shrimp, *Neotrypaea californiensis*, despite clear differences in growth. They hypothesized that *N. californiensis* may have adopted temperature independent metabolic pathways as suggested for other intertidal invertebrates that are commonly subject to strong fluctuations in their thermal environment (Newell 1969, Marshall & McQuaid 2011).

The burrowing shrimps, *N. californiensis* and *Upogebia pugettensis*, play an important ecological role in US west coast estuaries. Their burrowing activities oxygenate intertidal sediments, facilitating biogeochemical processes and influence community composition and the abundance of other invertebrates, epifauna, and infauna inhabiting the mudflats (Posey 1986, Posey et al. 1991, Ferraro & Cole 2007, D'Andrea & Dewitt 2009). They also have a negative impact on oyster aquaculture operations in the Pacific Northwest (PNW) which has led managers to seek economically and ecologically sustainable methods for their control on oyster beds (Dumbauld et al. 2006). As a result, managers are interested in developing age-based population dynamics models to help understand recruitment and mortality in these unique estuarine invertebrates across

varying spatial scales. Previous work has suggested that analysis of lipofuscin may provide a way to overcome the challenges of length-based age determination in *N. californiensis,* but these studies involved only short term experiments and did not attempt to characterize environmental influence on growth rate or lipofuscin accumulation (Coleman et al. unpublished data, Bosley & Dumbauld 2011).

Broad scale application of lipofuscin aging requires validation of the method and confirmation that lipofuscin provides a more robust estimate of age than body size across an array of environmental conditions (Tully & Fletcher 2000, Sheehy & Prior 2008). The purpose of this study was to test the hypothesis that temperature is a primary factor controlling growth rate and lipofuscin accumulation rate for two sympatric burrowing shrimp species, *N. californiensis* and *U. pugettensis.* We used a combination of laboratory and field experiments to evaluate the applicability of lipofuscin-based aging in species whose thermal and nutritional environments vary across spatial and temporal scales. We discuss recommendations for future aging studies and develop hypotheses regarding physiological interactions with the environment. This is the first study to explicitly test temperature effects on lipofuscin accumulation and growth rate in these west coast thalassinidean shrimps.

MATERIALS & METHODS

Mesocosm Temperature Experiments

A series of controlled experiments were conducted to determine the effect of temperature on growth and lipofuscin accumulation for both *N. californiensis* and *U. pugettensis*. Experiments took place in indoor mesocosms, located in a seawater laboratory at the Hatfield Marine Science Center, Newport, Oregon and each took place approximately 9 months. Mesocosms were constructed from 90 gallon Nalgene® plastic tubs equipped with continuously running raw marine seawater. The tanks were illuminated with fluorescent grow lights set to a 16hr/8hr light-dark cycle to represent summer light conditions. A total of 6 tanks were used for each trial which allowed for

three temperatures to be tested each with two replicates. Temperature treatments were 10° C, 13° C and 16° C which represent lower, average and upper temperatures commonly experienced by burrowing shrimp populations in Yaquina Bay, Oregon (Fig B1 in the appendix). Each of the six tanks contained 20 PVC pipes (10cm diameter x 40cm height) that were used to house individual animals during the experiment. Pipes were filled with sediment from sites where shrimp were collected and sieved with a 3 mm mesh to remove shrimp and other macrofauna.

Temperature of each tank was maintained separately with either a single (10 °C & 16 °C) or two-stage control system (13 °C). The temperature for the 16 °C treatment was maintained using a single 400-watt submersible heater. Inflow water was usually less than 16 °C and therefore no other temperature regulation was needed. The 13 °C treatment was maintained by both a 1/10 hp Ecoplus ® chiller unit and 400-watt submersible heater to keep the tank temperature at $13 \pm 1°C$ throughout the duration of the experiment. The 10 °C treatment was controlled by only 6 hp chiller as the water temperature coming into the tanks was usually ~ 12-13 °C, therefore no heating was needed for the cold treatment. Each tank contained a submersible pump to continuously circulate water during the experiments.

Shrimp for each trial were collected from Yaquina Bay, Oregon (Fig 3.1). *N. californiensis* were collected from a site (OSU Dock) with recent recruitment and assumed to be the same age based on body size (Dumbauld et al 1994, Bosley & Dumbauld 2011). Specific size range for Trial 1 was 8.41 ± 0.61 mm carapace length (CL) and Trial 2 was 10.71 ± 0.98 mm CL. *U. pugettensis* were also selected based on body size and assumed to be the same age at the onset of the study (13.72 ± 1.1 mm CL). Following collection from the field, shrimp were sexed and CL and weight recorded. Shrimp were then randomly assigned to tubes (20 in each tank) within treatments (3 with 2 reps) which included an additional set of samples being reserved as "initial" (n = 20). Shrimp were allowed to establish burrows and acclimate to the experimental mesocosms for 3 days before temperature treatments began. A single temperature trial for *U.*

pugettensis began on 12/7/ 2012 and lasted for 7 months until shrimp were sampled on 7/1/2013. Two temperature trials were completed for *N. californiensis*. The first began on 3/19/2014 and lasted until 11/25/2014 (8 mo). The second trial took place over the same time period the following year, beginning 4/23/2015 and ending on 12/11/2015 (8 mo). Temperature treatments were randomly assigned to tanks for each trial.

Following each temperature trial, shrimp were removed from the tanks and sex, CL and weight recorded. Animals were then rinsed in clean seawater and frozen at -80 °C until lipofuscin quantification could be done. To determine whether food conditions varied significantly among treatments during the second *N. californiensis* trial, surface sediment core samples were taken to a depth of 3 cm from randomly selected tubes within each tank at the termination of experiment. Sediment samples were used to determine sediment chl *a*, phaeopgiments and percent organic matter within tanks (see methods below).

Long-term field growth experiments

Long-term field growth experiments were conducted for both *U. pugettensis* and *N. californiensis* in Yaquina Bay, Oregon (44.6181° N, -124.0302° W) located on the central Oregon coast. The goal was to develop growth models for both species and confirm lipofuscin as a viable method for aging wild shrimp populations exposed to different environmental conditions. Furthermore, we qualitatively assessed food quantity as a potential factor contributing to variable growth among spatially separated populations of *N. californiensis*.

Upogebia pugettensis

Due to logistical constraints, growth experiments for *U. pugettensis* took place at one location on Idaho Flats (IF) in Yaquina Bay (Fig 3.1). Twelve large growth chambers were constructed from large plastic rubbish containers (~ 32 gallons). Bottoms of containers were removed and the sides drilled with small holes along their length to

ensure porewater flow and movement of organic matter. Growth chambers were placed in substrate within an established shrimp population and pressed down to leave 2 cm above the sediment surface. Sediments were sieved into the chambers with a 3 mm sieve to remove shrimp and other large macrofauna. Material within the chambers was allowed to settle for 2 days before animals were introduced.

Analysis of length data for *U. pugettensis* has shown that they can reach a size ranging from 11.23 – 15.23 mm CL over the first growing year (Dumbauld et al. 1996, Bosley unpublished) and we chose to use this 1 year old size class because they were abundant and small enough to re-establish a burrow. A total of 180 animals ranging in size from 11 – 15 mm CL were collected in May 2009. Fifteen animals were randomly assigned to each of the 12 growth tubes. Sex and carapace length were measured for each shrimp prior to placement in growth chambers. Containers were covered with a course mesh fabric for the first month to protect them from predation until burrows could be established. Water column temperatures were recorded at the OSU dock site using a YSI multiparameter water quality sonde operated and maintained by the EPA-Pacific Coastal Ecology Branch. Temperatures were recorded at a 15 min interval during the course of the experiment. Two growth chambers were randomly sampled every four months over a two-year period. During each sampling event, shrimp were collected by sieving the material in the growth chambers through a 3 mm sieve. All shrimp were measured for CL and frozen at -80 ° C until lipofuscin could be measured.

Neotrypaea californiensis

Measurement of growth and aging rate for *N. californiensis* was initially planned to take place at four locations along the estuarine gradient within Yaquina Bay (Fig 3.1). Two sites were designated as high intertidal sites (Sally's Bend (SB) & IF, + 2.0 mean low water (MLW)) and two sites were low sites (Seawall Island (SWI) & upriver (TI), + 0.1 MLW). At each location an array of 64 growth tubes was placed within the sediments. Growth tubes for *N. californiensis* were constructed of pvc pipes (10 cm

diameter x 46 cm height) that were fitted with 1 mm mesh bottoms to allow natural tidal flow into the tubes. A total of 480 ghost shrimp (240 males and 240 females) from a specific size range (4.8 ± 0.48 mm) were collected from the OSU dock in Yaquina Bay (Fig. B1). The animals were assumed to be age 1+ ghost shrimp based on size and assumed recruitment year (Dumbauld et al., in prep). After collection the shrimp were randomly assorted among the field growth chambers at density of two per tube with one female and one male introduced to each. Placing only one male and one female into each container allowed individuals to be identified on retrieval and potentially reduced any same sex competition within the tubes. Carapace length of all shrimp was measured prior to being introduced into the growth tube and mesh lids were placed on top of each container to keep immigration and emigration from contaminating the samples during the first year. All sediment in the tubes was sieved prior to being placed in the chambers to assure other shrimp and macrofauna had been removed. Temperatures were recorded in 30-minute time intervals at each site with HOBO temperature probes placed 10 cm below sediment surface.

Beginning one year after shrimp were placed in the tubes they were sampled every 6 months for a 2.5-year period. The SWI and TI locations were lost after one year due to sediment burial so only the SB and IF sites were sampled throughout the duration of the experiment. At each sampling event, 10 tubes were randomly selected and contents sieved (3 mm mesh) to remove shrimp. Sex, CL (mm) and wwt (g) were recorded prior to being stored at -80 °C until lipofuscin could be measured.

Measurement of environmental parameters

To model the effect of temperature on growth from field experiments, mean daily temperatures from the field study were converted to temperature growing degree-days (TDD) using the following equation (Curry & Feldman 1987, Puckett et al. 2008):

$$TDD \ (°C \ \cdot day^{-1}) = \sum_{i} \begin{cases} t_a - t_b, & if \ t_a > t_b \\ 0, & if \ t_a < t_b \end{cases} \tag{1}$$

where i is the number of days, t_a is the mean daily temperature (° C), and t_b is the basal temperature (10 °C) below which molting is thought to cease (Cassidy 2008). Values were not allowed to be negative, so if $t_a < t_b$, zero was added to the cumulative TDD total.

Sediment chl a, phaeopigment concentrations and percent organic matter were measured at each location with cores (3 cm x 5 cm) of surface sediments. A minimum of 6 cores were taken during each sample period, 3 inside shrimp tubes and 3 outside shrimp tubes. At IF, sediment samples were also taken from tubes not containing shrimp to compare sediment characteristics of empty tubes. Sediment chl a and pheaopigments were measured using spectrophotometric methods described by (Lorenzen 1967). Sediment organic matter content was determined using the loss-on-ignition method (Sutherland 1998, Heiri et al. 2001) where homogenized sediment samples were dried at 105 °C for 24 hours then weighed. Dried samples were heated in a muffle furnace 450 °C for 6 hours to burn off the organic material. Percent organic material was calculated as:

$$\text{Sediment \% organic material} = (DW\text{-}AFDW) \ / \ DW \tag{2}$$

where DW is the pre-ignition sample dry weight (g) and AFDW(g) is the post-ignition ash-free dry weight.

Measurement of lipofuscin

Lipofuscin concentration was determined with methods modified from Bosley and Dumbauld (2011). Brains were dissected, placed in a pre-combusted 1.5 ml amber vial and topped with 1.0 ml dichloromethane: methanol (2:1) solution. Samples were sonicated for 30 secs at 18% with a microprobe sonicator, and then stored in the freezer overnight to ensure complete extraction of lipofuscin. Samples were then evaporated to

dryness with pure N_2 and reconstituted with 0.25 ml HPLC grade methanol. Lipofuscin was measured with an Agilent 1100 scanning fluorescence detector at excitation wavelength 281 nm and emission wavelength 615 nm using methanol as a carrier solvent. Fluorescence peaks were maximized with a sample volume of 15 µl and a flow rate of 0.8ml min^{-1}. Lipofuscin concentration was quantified by calibrating fluorescence values to a standard of quinine sulfate in 0.1 N in H_2SO_4. Following lipofuscin measurement, samples were prepared for measurement of protein concentration by evaporating samples to dryness with pure N_2, and then reconstituting with 1 ml of 16% deoxycholic acid. Samples were sonicated in an ice-water bath sonicator for 30 min and stored in a refrigerator overnight before protein quantification. Protein concentration was measured with an Agilent 1100 fluorescence detector at excitation 280nm and emission 345 nm using nanopure water as a carrier solvent. Peaks were maximized with a 12 µl sample volume and 0.8 ml min^{-1} flow rate. Fluorescence intensity of extracted protein was calibrated with a standard of bovine serum albumin (BSA) in 16% DOC (Harvey et al. 2010, McGaffin et al. 2011). Lipofuscin concentration was normalized to protein concentration in each sample to account for differences in body size and variability in brain tissue dissections. The lipofuscin based age metric was termed lipofuscin index (LF Index):

$$\text{LF Index ng µg}^{-1} \; = \; \frac{\text{lipofuscin conc ng ml}^{-1}}{\text{protein conc µg ml}^{-1}} \tag{3}$$

Statistical analysis

Mesocosm experiments

Growth was calculated as the change in mean carapace length (Δ CL) for each tank from experiment start to end. Shrimp grown within a single tank were not statistically independent so to avoid pseudo-replication in mesocosm temperature experiments tank was treated as the experimental unit. Effect of temperature treatment

on growth was tested with Analysis of Variance (ANOVA) and trial was also included as a predictor variable for *N. californiensis*. Similarly, mean LF Index was calculated for each tank and difference among treatment tested with ANOVA using mean change in LF Index (Δ LF) from each tank as the dependent variable with treatment and trial as predictor variables.

The second temperature trial for *N. californiensis* was improved over the first by applying lids to tubes within the tanks to retain animals throughout the course of the experiment. This improvement made it possible to track individual growth and changes in the allometric (length:weight) relationship. The overall change in the allometric relationship for *N. californiensis* was examined with a linear mixed-effects model using the LME4 package in R (Bates et al. 2015). Log transformed shrimp wwt (g) was predicted as a function of the fixed-effects of body size (CL) and time (initial vs. final) and tank was set as a random variable to account for individuals grown in the same tank. Statistical significance of the time parameter was tested by comparing a full model containing all predictor variables with a reduced model which included only CL as a fixed effect using a sums-of-squares F-test.

Field growth experiments

N. californiensis

Individual growth for *N. californiensis* was calculated as the change in carapace length (Δ CL) over the time the shrimp were in growth tubes. There were several cases at both IF and SB where smaller shrimp were found in tubes and likely arrived as recruits. These presumably younger shrimp were omitted from the analysis. LF index values were log-transformed to meet the assumptions of normality and equal variance.

A model selection procedure was used to test the relationship between growth and lipofuscin accumulation with time, temperature-degree days (TDD), location and sex. A series of multiple linear regression models were constructed that included, time (days elapsed) or TDD, location and sex. If sex was not found to be a significant explanatory

variable, Δ CL and log (LF Index) were averaged within tubes to avoid pseudo-replication as shrimp from the same tube were not independent. The best model to describe growth and lipofuscin accumulation rates were determined by backwards model selection and comparison of adj R^2, Akaike's Information Criterion (AIC) and Bayesian information criterion (BIC) values. Growth differences were qualitatively related to sediment characteristics at each location. Differences in sediment chlorophyll and % OM were determined with ANOVA using location, time and sample type as predictors.

U. pugettensis

Length frequency data of *U. pugettensis* sampled from field enclosures clearly showed recruitment of new individuals. Cohorts were visually distinguishable and were divided statistically using the mixdist package in R (Du 2002, Macdonald 2015). The mixdist package provides estimates for mixing proportions, mean, and standard deviations by fitting finite mixture distribution models to frequency histograms (Macdonald 2015). Because multiple cohorts were present in *U. pugettensis* enclosures, CL-at-time measurements were used to model growth for each cohort instead of Δ CL as was done with *N. californiensis.* Backwards model selection was used to determine the relationship of size with time (days elapsed), temperature-adjusted time (TDD), cohort and sex. CL measurements were averaged for each cohort within buckets to avoid pseudo-replication of individuals of the same age living in the same bucket. LF index values were only available for the last two sampling times because earlier samples were lost in a freezer failure, but each remaining sample included several age groups as determined by size. Age was predicted using the best growth model and LF index value was then related to size-based age, sex and sample date with multiple regression. The best lipofuscin model was selected based on adj R^2, AIC and BIC values. All statistical analyses were conducted in the software package R (R development Core Team 2013).

RESULTS

Mesocosm experiments

Body sizes for both *N. californiensis* and *U. pugettensis* were not statistically different among tanks at the onset of the trials (ANOVA, *N. californiensis;* trial 1 $F_{6, 107} =$ 0.159, p = 0.987, trial 2 $F_{6, 133} = 0.668$, p = 0.675, *U. pugettensis;* $F_{6, 140} = 0.825$, p = 0.553*).* Following the experiment, there was no difference in mean Δ CL among temperature treatments for *U. pugettensis* (ANOVA, $F_{2,3} = 0.328$, p = 0.743) or *N. californiensis* (ANOVA, $F_{3,8} = 1.024$, p = 0.430) after accounting for difference between trials (Fig 3.2A & 3.3A, Table 3.1). LF Index clearly increased in *U. pugettensis* over the 7 month experiment, but accumulation was not influenced by temperature (Fig 3.2B; ANOVA, $F_{2,3} = 0.785$, p = 0.56) with initial LF Index increasing 65.7 % from 3.99 ± 1.43 ng µg^{-1} to an average (pooled) LF index of 6.066 ± 1.27 ng µg^{-1}. Lipofuscin values increased for *N. californiensis* as well, but accumulation was influenced by temperature (Fig 3.3B). The initial samples from trial 2 were lost in a freezer failure, but we assumed that initial values from trial 1 were comparable for trial 2 and used trial 1 initial values to calculate Δ LF Index. After accounting for differences among trials, Δ LF Index in the 16 °C treatment was significantly less than in the 13 °C or 10 °C treatments, which were not statistically different from each other (Table 3.1, $F_{3,8} = 9.88$, p = 0.004).

The allometric relationship for *N. californiensis* changed significantly over the course of the experiment (Fig 3.4, Table 3.2). The linear mixed effects model indicated shrimp were 18.9% lighter on average after the experiment than when they were introduced to the tank at the beginning. This was likely because of the poor food conditions in the tank. Chl *a* concentrations were relatively low averaging < 1 mg g $^{-1}$ and % organic matter was also low (<1%, Table 3.3).

Field growth experiments

N. californiensis

Temperature degree-day values were slightly higher at the IF site during the first summer growing season (Fig 3.5). This was likely because mean daily temperatures at SB tended to be less variable than at IF. Analysis of growth rates for *N. californiensis* indicated that sex was not a significant factor in predicting ΔCL therefore tube means were calculated for models containing the variables time and site. Both TDD and days elapsed were important predictors of growth for *N. californiensis* in Yaquina Bay. The best model included TDD and site as well as the interaction between the two variables and described 51% of the variance in the data (Table 3.4). Parameters from the model indicated that average growth rate was significantly higher at the SB location than IF (Fig 3.6, Table 3.5). Growth rate was estimated to be 0.0007 ± 0.0002 mm TDD^{-1} or ~ 0.70 mm yr^{-1} at IF and nearly twice that at SB (0.0014 ± 0.0005 mm TDD^{-1} or ~ 1.4 mm yr^{-1}), but there was considerable overlap in the data. LF Index increased at both locations and accumulation was best described by days elapsed but was only slightly better than a model containing TDD (Table 3.4) based on AIC. There was a weak effect of site with LF Index values being slightly lower at SB location relative to IF but lipofuscin accumulation rate was consistent across sites (Table 3.5, Fig 3.6B). LF index increased by approximately 1.0012 % day^{-1}, (95% CI; 1.0008%-1.0016% day^{-1}) corresponding to annual increase of 54.9 % yr^{-1} (95% CI; 34.3%-78.8% yr^{-1}) but there was considerable overlap in the data.

Sediment characteristics were significantly different between sites (Table 3.6, Fig 3.7). Organic matter was lowest at IF (~ 1 %) and increased with distance from the estuary mouth (Tukey's post-hoc pairwise comparisons, $p < 0.001$). Sediment at the SB site had approximately three times the amount of organic matter than measured at IF. Sediment at SB was rich in phytopigments relative to IF with approximately twice the amount of chl *a* and phaeopigment (Table 3.6). Phytopigments and % organic matter were significantly higher in tubes from IF where shrimp were not present (Table 3.6,

Tukey's post-hoc pairwise comparisons, p < 0.0001). This result suggests that shrimp influence the abundance of algal material within the tubes, probably through feeding.

U. pugettensis

Modal analysis indicated four cohorts present in *U. pugettensis* field enclosures (Figure 3.8). Several very large shrimp were found in buckets during the early sampling events and it was assumed that these were older shrimp that burrowed through the bottom of the enclosures and were omitted from the analysis. Growth models indicated sex was not a significant predictor of CL in *U. pugettensis* (Table 3.7) therefore mean CL was calculated for all individuals of the same cohort from the same bucket to avoid psuedo-replication. The best model describing growth included cohort, age in days and their interaction as predictors and described 91.5% of the variability in CL. Parameters of the best model estimated growth rate in the original cohort to be 0.018 ± 0.0014 mm day^{-1}, which is approximately ~ 6.6 mm yr^{-1}. Growth rates increased in subsequent cohorts with growth in the second cohort estimated to be ~ 8.5 mm yr^{-1}. A similar model which included TDD and cohort as predictors and the interaction indicated either weak difference or no differences in slopes among cohorts (Table 3.7) providing evidence that variable growth among cohorts may be related to inter-annual temperature differences. Growth rate for the TDD model was estimated to be 0.013 ± 0.001 mm TDD^{-1} or ~ 7.10 mm yr^{-1} and a plot overlaying temperature-degree days tracked well with carapace length for the primary cohort in field enclosures (Fig 3.9A). Because the TDD model showed little difference in growth across cohorts, it was used predict temperature-adjusted ages of shrimp sampled from the last two time steps for correlation of LF index with age (Fig 3.9B).

LF Index in *U. pugettensis* did not vary with sex, predicted age or cohort but was best described by sample date (Fig 3.9C). Shrimp sampled at 751 days (6/8/2011) had a mean LF index of 5.9 ± 0.32 ng μg^{-1} (Table 3.7). Mean LF index for shrimp sampled at the end of the experiment (932 days, 12/6/2011) was significantly lower than shrimp

sampled at 751 days (3.9 ± 0.455 ng µg^{-1}) even though shrimp had clearly grown between the two time steps.

DISCUSSION

Mesocosm experiments

It is well know that ambient temperature is a determinant of metabolic rate in poikilotherms with metabolism generally increasing with increased temperature (Gillooly et al. 2001). Previous studies describing temperature effects on lipofuscin in crustaceans have shown elevated temperatures cause an increase in accumulation rate (Sheehy 1990, 2002, Sheehy et al. 1995, O'Donovan & Tully 1996a, Tully & Fletcher 2000). However, our laboratory experiment showed accumulation in *N. californiensis* was depressed in the high temperature treatment and this trend was consistent over two trials. Depression of metabolic rate at high temperature has been shown to occur in other aquatic poikilotherms (Newell 1969, Newell & Branch 1980, Marshall & McQuaid 2011). At temperatures above normal, physiological processes are affected which may include reduction of oxygen transport, changes in membrane permeability, denaturation of proteins and enzyme thermal optima may be exceeded (Prosser 1973). Together these physiological responses may result in lower lipofuscin accumulation rates at thermal extremes.

The high temperature treatment of 16°C was selected because it is approaching the upper limit for sediment temperatures that burrowing shrimp experience in Yaquina Bay (Figure B1). While shrimp commonly experience temperatures in this upper range, it is unlikely that they would suffer prolonged exposure at these elevated levels. Our study showed that LF index was not statistically different between *N. californiensis* reared at 10 °C and 13 °C. These results suggest that temperature influence on lipofuscin accumulation may be minimal within the normal temperature range that shrimp populations experience in the field, providing further evidence for the broad scale applicability of lipofuscin-based aging for ghost shrimp. While lipofuscin accumulation

rate is clearly affected by high temperatures in *N. californiensis,* the same affect was not observed for *U. pugettensis.* The low number of replicates in our temperature trial did not provide enough power to detect statistical differences in lipofuscin accumulation across treatments, but qualitatively there was little difference in LF Index values at the end of the experiment. This trend suggests that lipofuscin accumulation may not be influenced by temperature in *U. pugettensis* but additional studies with an increased number of replicates should be conducted to confirm these findings.

LF Index for both species increased relative to the initial samples despite little to no change in body size and in several cases we documented negative growth (shrinking). An unexpected result of the laboratory mesocosm temperature trials was the change in the allometric length-weight relationship over the duration of the experiment. Body size in *N. californiensis* was not affected by temperature but 40% of shrimp were observed to shrink and almost all (93%) lost weight over the 9-month period. Despite these changes, survival rates were relatively high (~ 90%). Food quality and temperature have been shown to affect both molt increment and intermolt period in a number of crustaceans species, which can lead to large variation in individual size-at-age and development rates (Hartnoll 1982, 2001, Oh & Hartnoll 2000). A few studies have also shown crustaceans have the ability to shrink, most of which have focused on the krill species, *Euphausia suberba* (Ikeda & Dixon 1982, Nicol et al. 1992, Marinovic & Mangel 1999). These studies proved that krill can molt to smaller sizes during periods of food limitation (Ikeda & Dixon 1982, Nicol et al. 1992) or elevated temperature (Marinovic & Mangel 1999). There are clear ecological advantages to being able to shrink when environmental conditions are poor. Metabolic and nutritional requirements for smaller animals are less which allows more resources to be allocated to maintenance of metabolic processes and survival. Phytopigment concentrations and % sediment organic matter in mesocosm tanks indicated that food levels were very low but % organic matter was consistent with field samples from the IF study site where ghost shrimp tend to be relatively small in size (Chapter 2). Shrinkage in estuarine burrowing shrimp populations would significantly

alter population size structure and de-couple the relationship between size and age. This has been observed for *N. californiensis* populations in Yaquina Bay, Oregon and other estuaries along the US west coast (Bosley & Dumbauld 2011, Coleman et al. unpublished data).

Mud shrimp survival was much lower in temperature trials than *N. californiensis* (~ 30%). Feeding studies on *U. pugettensis* have shown their diets to be mainly comprised of marine derived phytoplankton and algae (Chapter 2). While the water being drawn into mesocosms was raw marine water from the bay, it passed through a settling tank before being introduced to experimental treatment tanks. It is likely the phytoplankton concentration in this raw marine water was not sufficient to sustain growth and survival in the tanks. Several mud shrimp (~9%) shrank over the 9-month experiment while others stayed the same size or did not survive suggesting food limitation may be important source of mortality in *U. pugettensis***.**

Field experiments

Data from field experiments provided evidence that environmental influences (i.e. temperature and food) affect growth in burrowing shrimp. For *N. californiensis,* growth was best modeled as a function of TDD and indicated growth rate at the SB location was twice that observed at IF. By the final sampling period the IF site had accumulated 418 more TDD than SB. This divergence seemed to occur during the first growing season but tracked fairly closely thereafter. Food differences between the two locations were more striking with sediment organic matter and phytopigments elevated three-fold and two-fold at SB over measurements taken at IF. Food quality and quantity can have dramatic effects on crustacean growth rates (Oh & Hartnoll 2000, Hufnagl & Temming 2011, McLean & Todgham 2015). McLean and Todgham (2015) investigated food effects on growth in the juvenile Dungeness crab, *Metacarcinus magister*, and found crabs fed under low food treatments had significantly fewer molts than well fed crabs. Data presented in Chapter 2 of this dissertation determined spatial patterns in food sources

available to burrowing shrimp in Yaquina Bay. It was hypothesized that shrimp populations with greater availability of high quality foods would experience elevated growth rates. Our data supports this hypothesis for *N. californiensis* and provides further evidence that the size-age relationship is site specific.

Lipofuscin accumulation rate in *N. californiensis* was constant across sites despite clear differences in growth rate. LF Index values were slightly higher at IF and this may have been because shrimp were placed in tubes at IF approximately 2 months before the experiment at SB began. Shrimp for SB were selected at the same size class at IF but because it was two months later (Sept vs. July), they may have been slightly younger animals at the time they were introduced to the experiment. We also found that modeling lipofuscin accumulation as a function of TDD did not significantly improve prediction of LF index. Previous studies have shown lipofuscin accumulation rate to vary seasonally with changes in water temperature (Tully & Fletcher 2000, Kodama et al. 2006, Puckett et al. 2008). Generally, lower temperatures cause a depression in overall metabolic rate during winter months, but this pattern may not apply to all organisms. Some aquatic invertebrates have the ability to maintain constant metabolic rate over a range of food conditions and temperatures by diverting energy away from growth and towards maintenance during times of thermal and nutritional stress (Sokolova et al. 2012). This function has been demonstrated in a number of intertidal invertebrates that are routinely exposed to dramatic fluctuations in environmental stressors (Newell 1969, Newell & Branch 1980, Marshall et al. 2011). Similarly, *N. californiensis* may have adapted mechanisms to cope with their highly variable environment by maintaining a constant metabolic rate while re-allocating energy to different physiological processes (ie. growth and reproduction) as conditions allowed.

Lipofuscin accumulation rate for *N. californiensis* in the field was estimated to be approximately 43% yr^{-1}. A similar study was conducted to establish lipofuscin accumulation rate in *N. californiensis* reared in outdoor mesosocms from known age animals (Coleman et al, unpublished data). This work estimated a linear accumulation

rate of ~ 1.4 ng µg^{-1} yr^{-1} which is comparable to the rate presented in this study (Fig 3.10). Our field experiments, however, showed a wide range of lipofuscin values at each age. Individual variation in lipofuscin accumulation has previously been shown within animals of the same age may reflect genetic and physiological differences among individuals (Sheehy 1990, Sheehy et al. 1995, O'Donovan & Tully 1996b, Wahle et al. 1996). It is also possible that some variation in LF index values may have been because animals we put into the tubes were not all the same age. We purposely selected animals in a narrow size class from the same locations hoping to ensure experimental animals represented a single cohort, but variation in size-at-age may begin at young age/size classes. Additionally, the tubes used to house the shrimp during the experiment did not have lids following the first year allowing the possibility for movement of outside shrimp into the tubes. Shrimp within the tubes were on average smaller than the surrounding population so if immigration did occur it was likely the result of juveniles moving in. Future aging studies should investigate tagging methods or employ techniques to more easily track individuals over the course of study and minimize extraneous sources of variation.

Field experiments showed growth rates in *U. pugettensis* could be explained by both calendar time and TDD, but data showed clear temperature effects on growth. Modelling growth as a function of temperature-degree growing days is becoming more widespread in ecological studies but has been largely overlooked in fisheries (Neuheimer & Taggart 2007). The TDD metric provides a way to model temperature related physiological processes (i.e. growth) and simplifies models by reducing parameters and linearizing the response (Neuheimer & Taggart 2007). Calendar time was a strong predictor of size for *U. pugettensis*, but modeling growth as a function of temperature-varying time showed that variation in growth among cohorts was largely a result of annual differences in the thermal environment, as was evident in the consistent slopes. The strong predictive power of TDD in estimating size in *U. pugettensis* suggests age can be robustly estimated as long as there is a temperature record available. Based on

calendar time, we estimated growth in *U. pugettensis* to be approximately 7 mm yr^{-1}. This rate is slightly higher than the growth rate reported for *U. pugettensis* in Willapa Bay, which was estimated to be ~ 4-5 mm yr^{-1} (Dumbauld et al. 1996). Our growth study for *U. pugettensis* took place at one location within Yaquina Bay. Further studies should be completed to determine how the TDD model holds up across other populations. Mud shrimp were not observed to grow in mesocosm temperature trials and this was likely because food conditions in our mesocosm temperature trial was not sufficient to induce growth. As seen in other crustacean species, food quality and food quantity effects may interact with temperature to cause a variable growth response in (Hardy & Duncan 1994, Paglianti & Gherardi 2004, McLean & Todgham 2015), which would require growth models for *U. pugettensis* to be constructed on a site-by-site basis.

While field data showed CL in *U. pugettensis* to display a positive linear relationship with time, we did not see a significant relationship between estimated age and LF Index. The lipofuscin compound is ubiquitous in all eukaryotic cells (Katz et al. 1984, Sheehy et al. 1990), but it does not always correlate directly with age (Medina et al. 2000, Allain et al. 2011, Crowley et al. 2014). Intra-cellular lipofuscin accumulation results from a build-up of worn-out or damaged cell components (Terman & Brunk 1998). In the case of short-lived and fast growing species such as *U. pugettensis*, the rate of anabolism (production of new tissues) may overtake the rate of catabolism resulting in a dilution of intracellular age-pigment (Terman 2001, Philipp et al. 2005). This process of "rejuvenation" can occur during intensive growing periods when animals are young or periods of high regeneration rates such as during reproduction (Terman 2001). Sukhotin et al. (2002) found that accumulation of fluorescent age pigments in the mussel, *Mytilus edulis,* increased exponentially with age, but was also negatively correlated with mussel size (lifetime growth rate). These results suggest that age and size effects on the physiological functions of continuously growing species can act in opposite directions to mask each other. Lipofuscin concentration in *U. pugettensis* was correlated with body size (Fig B2), but when normalized to tissue concentration, the relationship with age did

not hold. Instead, LF index was predicted by sample date with higher ratios found in shrimp collected during the summer. Changes in the LF Index with time suggest that growth process and possibly extrinsic environmental variables, such as salinity or oxidative stress, may influence the ratio of intracellular lipofuscin to total brain tissue protein during certain times of the year. Additional studies should be conducted to determine factors driving temporal changes in LF Index for *U. pugettensis* and how those changes relate to the metabolic balance of anabolism and catabolism in the organism.

Lipofuscin aging provides a viable alternative for estimating age in crustaceans where length measurements do not correlate to size. However, broad scale application of the method requires that accumulation rates are not greatly influenced by extrinsic environmental factors which can vary greatly over time and space. The results presented here support the hypothesis that analysis of lipofuscin provides a more robust estimate of age than size in the ghost shrimp, *N. californiensis*, whose growth is influenced by variation in food availability and to a lesser extent, temperature. Individuals may grow, shrink or remain the same size over long periods leading to a de-coupling of the age-length relationship while lipofuscin accumulation rate is conserved over a range of food and temperature conditions. Conversely, data from this study did not support this hypothesis for *U. pugettensis*. Body size measurements were tightly correlated to time and age could be predicted by accounting for thermal history. Lipofuscin was present in *U. pugettensis,* but given the strong relationship between size and age, we recommend using size as a proxy for age in conducting demographic assessments of mud shrimp. This is the first study to demonstrate how environmental variability can influence growth and aging rates for burrowing shrimps in US PNW estuaries and provides evidence for broad scale application of aging methods for both species which can be applied to assess their population dynamics.

ACKNOWLEDGEMENTS

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Figure 3.1 Map of Yaquina Bay, Oregon, showing planned study sites and collection site for field growth experiments. Red locations (SWI; Seawall Island and TI; Tongue Island) were lost after the first sampling. IF; Idaho Flats, SB; Sally's Bend. Mud shrimp study took place at IF and *N. californiensis* for the indoor mesocosm experiment and tube transplanting study were collected at the OSU dock site.
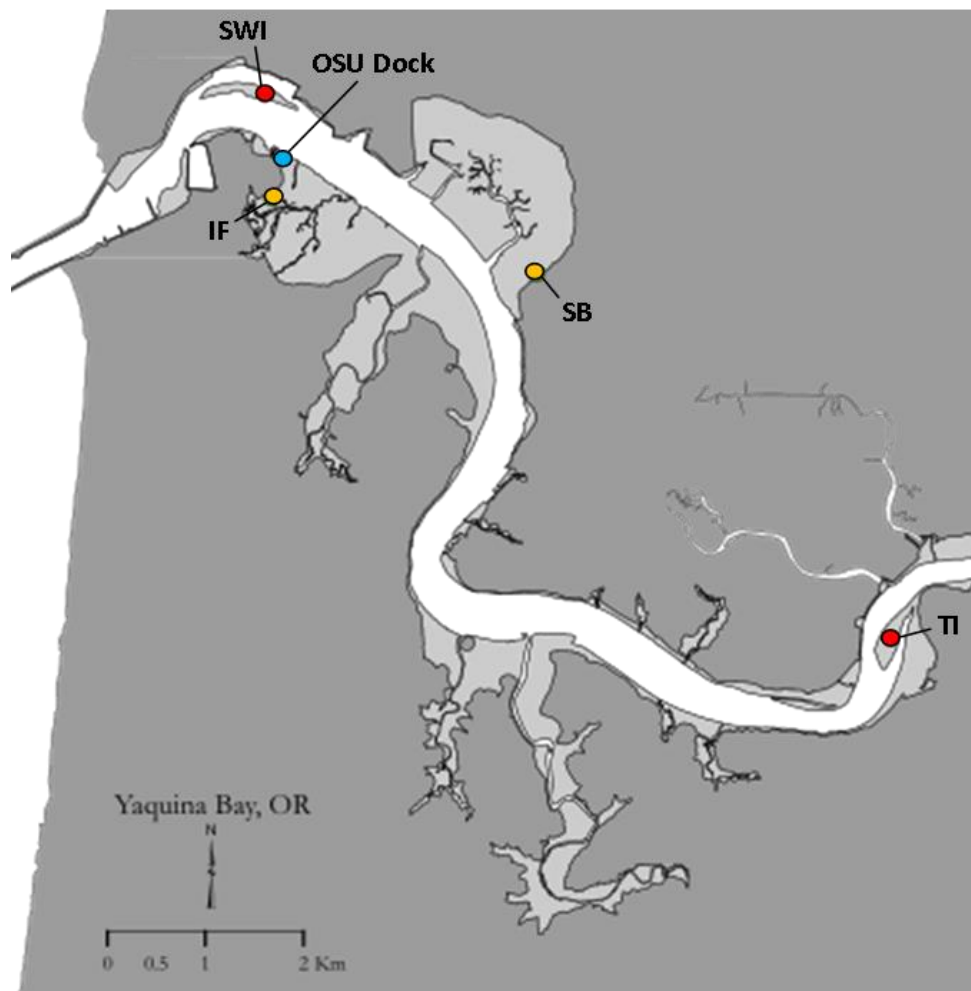
Figure 3.2: Results showing Δ CL and Δ LF Index for *U. pugettensis* from mesocosm temperature trial. A) barplot showing mean carapace lengths of shrimp from each temperature treatment before and after the experiment, B) barplot showing mean LF Index values from the onset of the experiment (initial) to the end for all three temperature treatments. Error bars represent within treatment standard deviations.
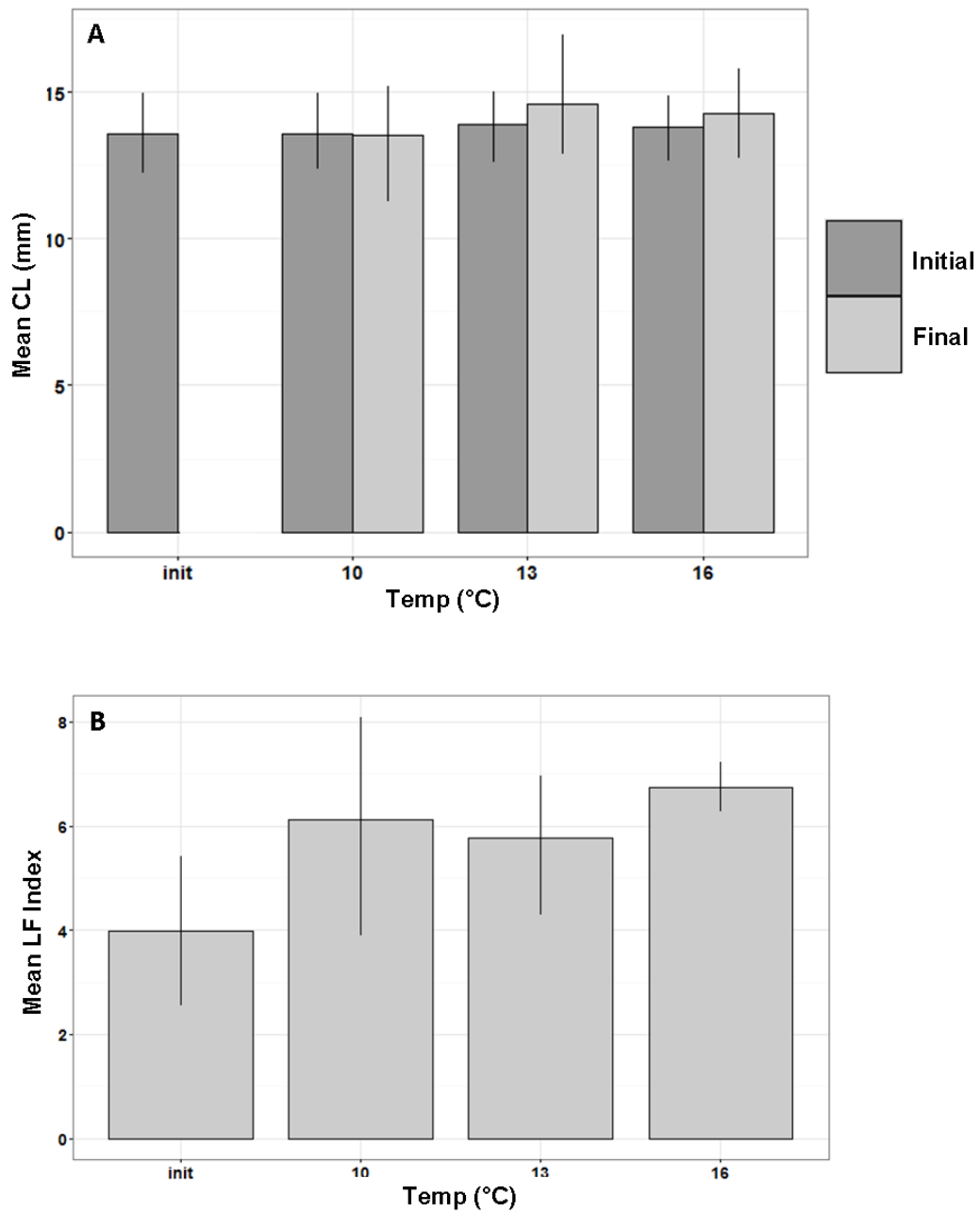
Figure 3.3 Results showing Δ CL and Δ LF Index from *N. californiensis* mesocosm temperature trials, A) barplot showing mean carapace lengths of shrimp from temperature treatments before and after each trial, B) barplot showing mean LF Index values measured at the onset of the experiment (initial) and end for all three temperature treatments. Initial samples were lost in trial 2 because of a freezer failure. Error bars represent within treatment standard deviations
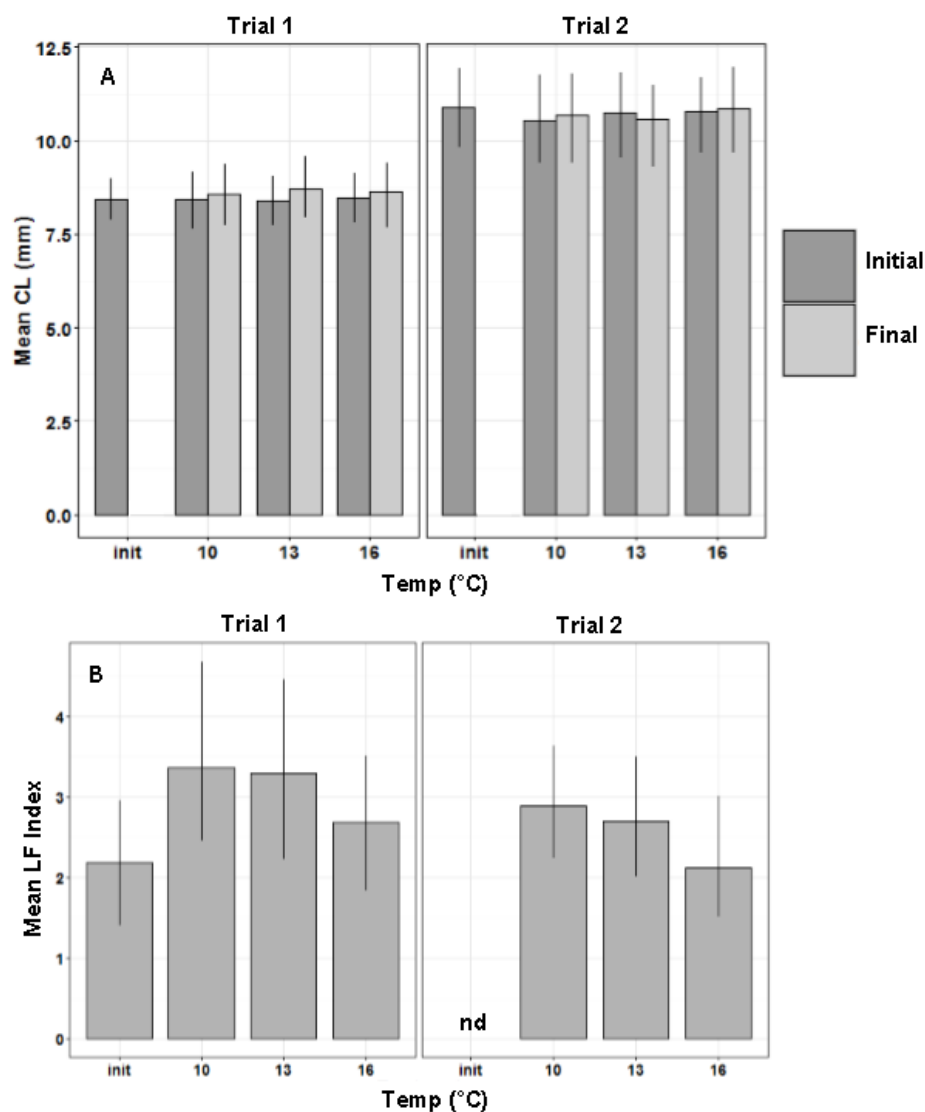
Figure 3.4 Change in allometric (length-weight) relationship for *N. californiensis* from temperature mesocosm temperature trial 2. Relationship at onset of study is shown in blue and final is shown in red. Treatment temperatures are labeled with different symbols.
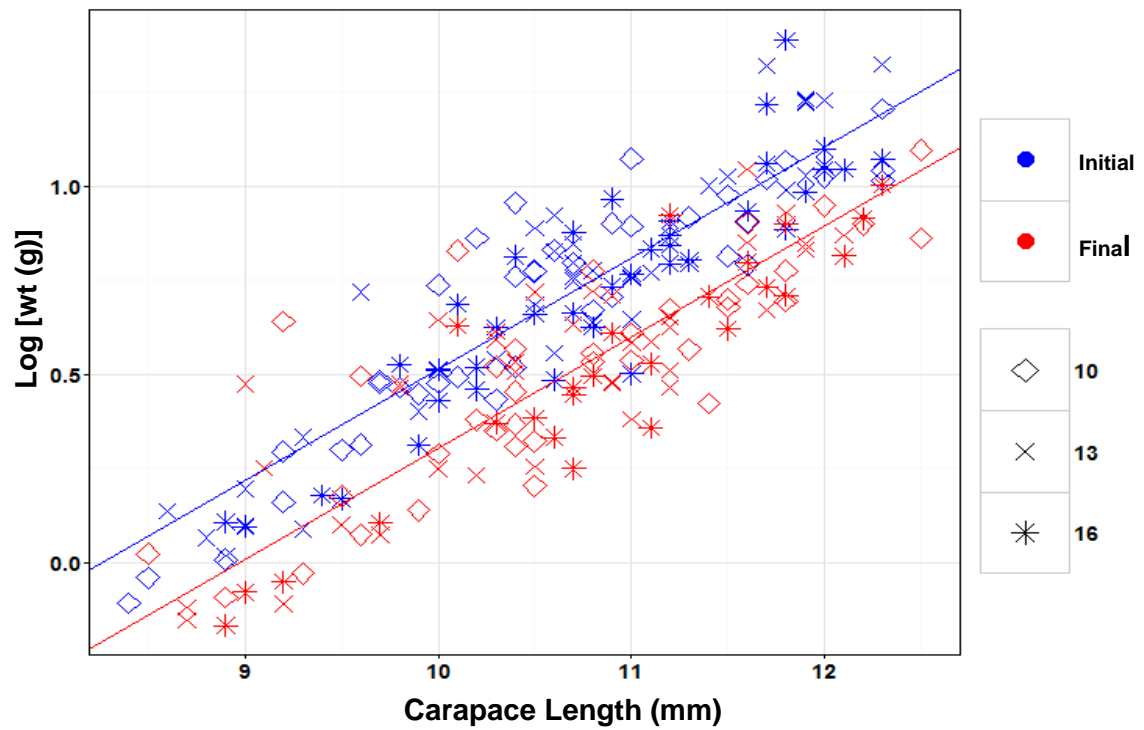
Figure 3.5 A) Plot of cumulative temperature degree-days calculated for field locations where growth experiments took place. B) Plot showing the difference in mean daily temperatures between locations. Line on plot represents the 1:1 relationship. IF; Idaho Flats, SB; Sally's Bend
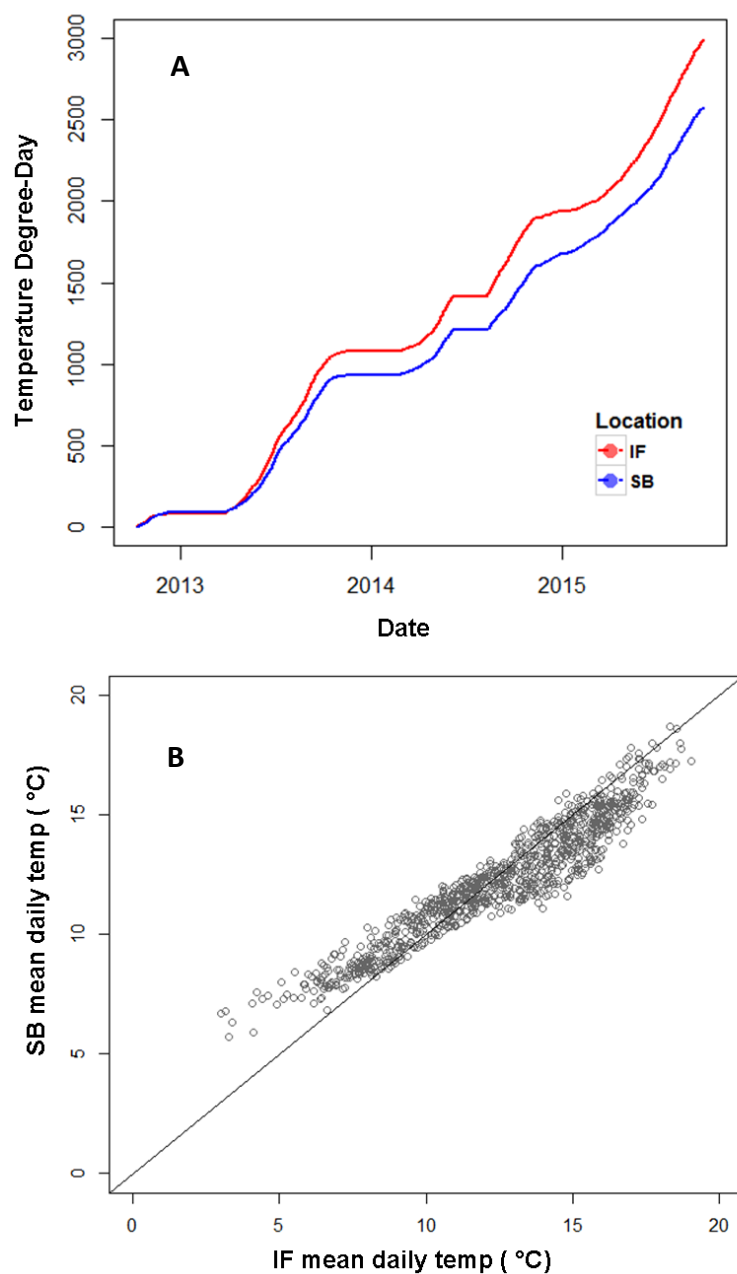
Figure 3.6 A) Change in carapace length (CL) in relation to temperature-degree days (TDD) for *N. californiensis* collected from tubes at field study locations. B) LF accumulation rate in relation to chronological time for *N. californiensis* determined from field growth experiment. Regression lines represent estimated growth rates based best fit model from model selection procedure. IF; Idaho Flats, SB; Sally's Bend
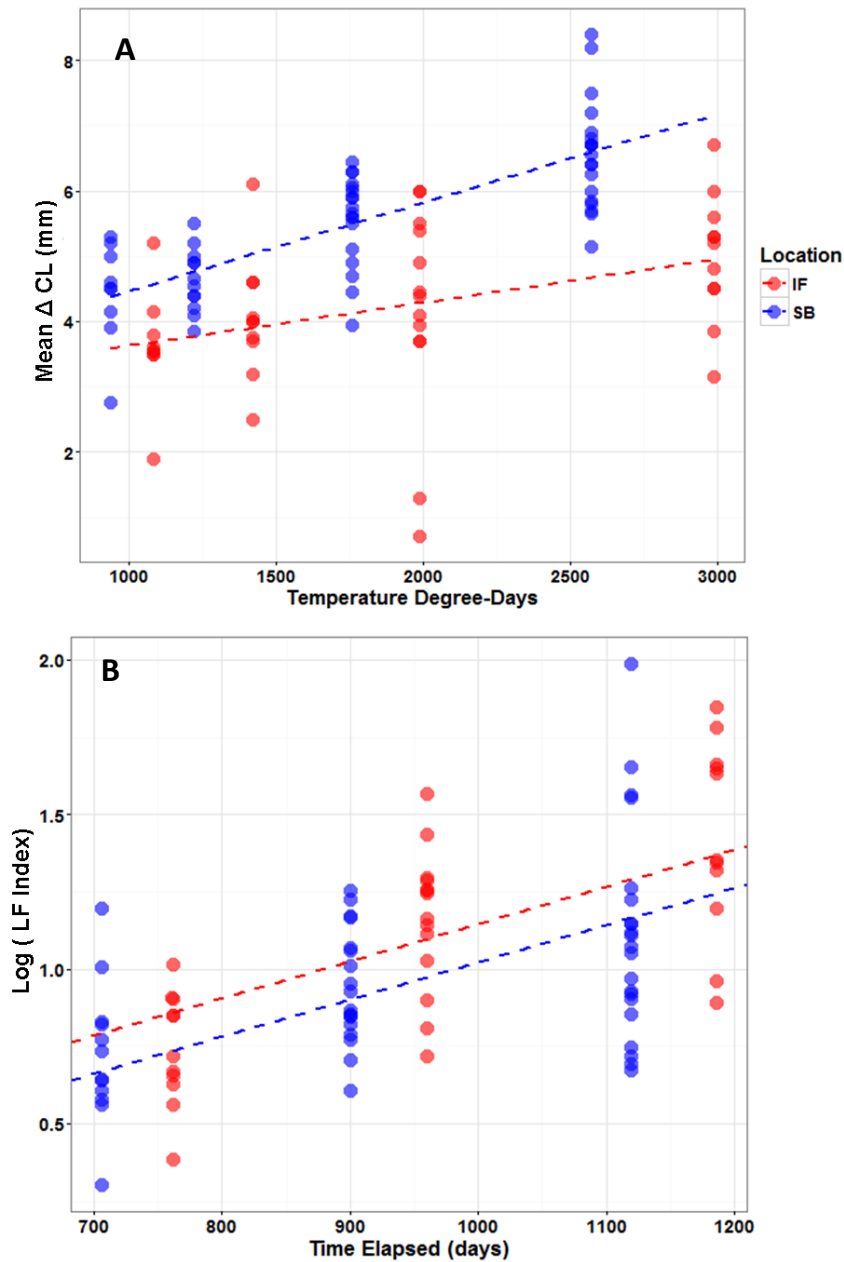
Figure 3.7 Sediment characteristics of field growth locations. A) % organic matter for three locations. Data is pooled over sample times. B) Sediment Chlorophyll *a* and phaeopigment concentrations at field locations. Different letter notations indicate significant differences (Tukey's pairwise comparisons, p < 0.05).

95
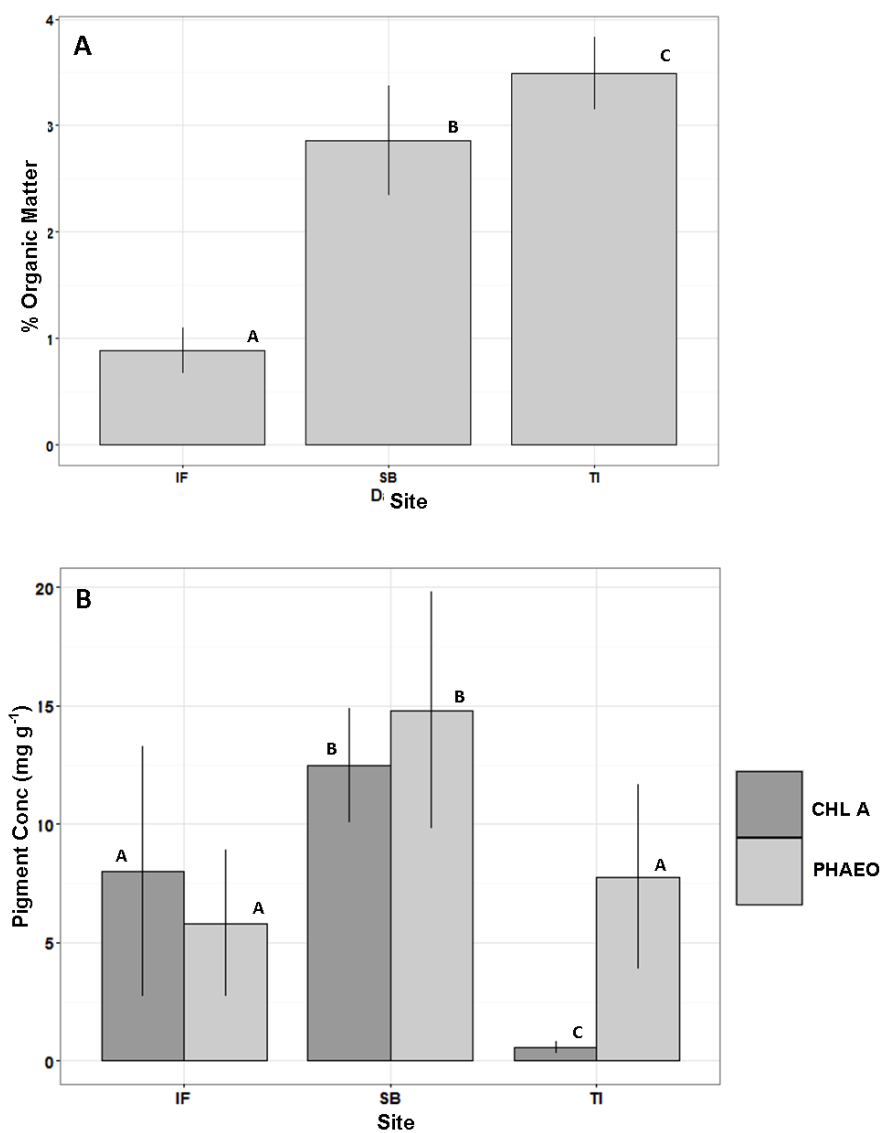
Figure 3.8 Length frequency histograms for samples of *U. pugettensis* collected from field growth experiment over time. Different color bars represent cohorts determined with modal analysis. White bars show the larger, presumably older shrimp that were omitted from the analysis. CL; Carapace Length (mm)
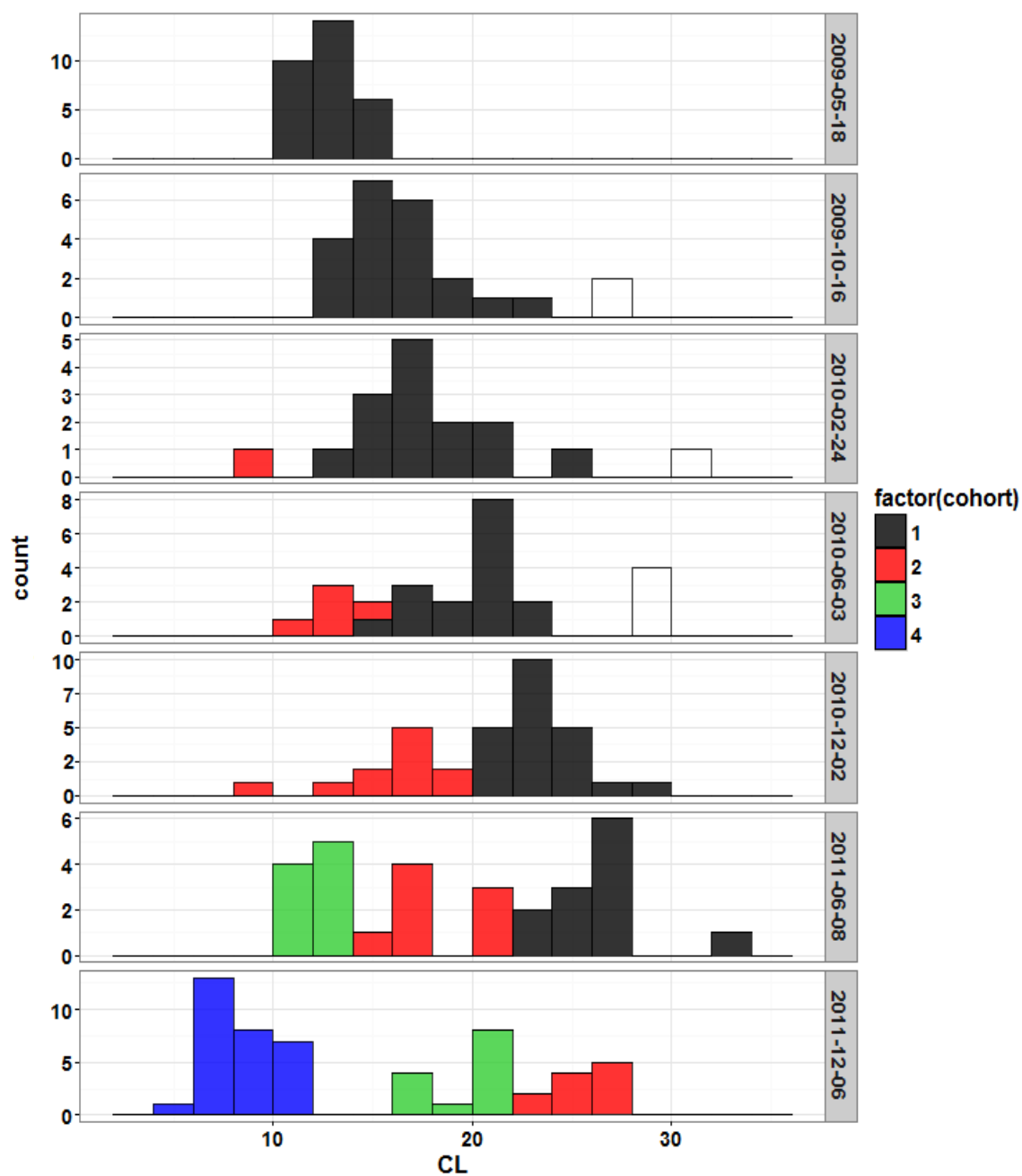
Figure 3.9 A) Plot of change in carapace length (CL) of original cohort in relation to sample date for *U. pugettensis* from field growth experiment on Idaho Flats. Symbols denote sex and overlay shows temperature degree-day (TDD) B) Results of multiple regression for best model fit of *U. pugettensis* growth in relation to TDD. Colors indicate the cohorts determined by modal progression analysis of CL frequency data from field enclosures. C) Plot showing relationship of LF Index to predicted age (TDD) estimated with size-based growth model. Different symbols represent sample periods (days elapsed), each comprised of multiple cohorts.
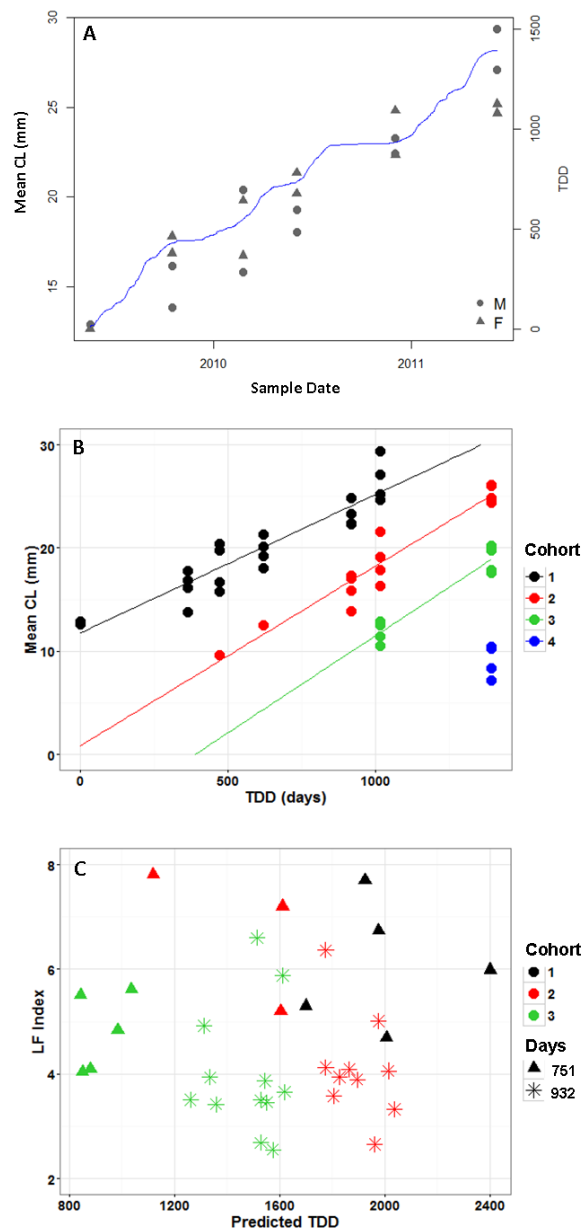
Figure 3.10 Best lipofuscin-age model for *N. californiensis* showing average, high and low accumulation rates (54.9 % yr^{-1}, 95% CI; 34.3%-78.8% yr $^{-1}$) determined from field growth study. Red line represents the linear LF accumulation rate presented in Coleman et al (unpublished data) estimated from mesocom study of known age shrimp (1.523 $\pm$ 0.046 ng μg yr-^{1}).
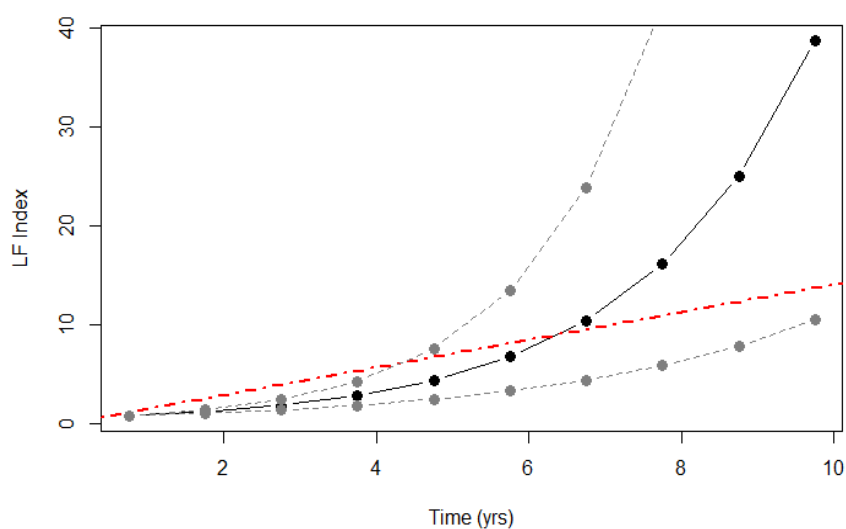
Table 3.1 Tables showing results for Analysis of Variance (ANOVA) comparing change in carapace length (CL) and change in LF Index across temperature treatments for both *N. californiensis* and *U. pugettensis*. Statistical differences are p < 0.05.

U. pugettensis				
Carapace Length				
Coefficient	**Estimate**	**SE**	**t**	**p**
Intercept	0.167	0.445	0.374	0.733
13	0.442	0.630	0.702	0.534
16	0.442	0.630	0.702	0.534
			$F_{2,3}$	0.328
			adj R^2	0.000
			p	0.740
LF Index				
Coefficient	**Estimate**	**SE**	**t**	**p**
Intercept	2.130	0.451	4.732	0.042
13	-0.352	0.638	-0.552	0.636
16	0.626	0.781	0.802	0.507
			$F_{2,3}$	0.785
			adj R^2	0.000
			p	0.560

N. californiensis				
Carapace Length				
Coefficient	**Estimate**	**SE**	**t**	**p**
Intercept	0.428	0.204	2.098	0.069
13	-0.083	0.144	-0.574	0.582
16	-0.011	0.144	-0.075	0.942
Trial	-0.193	0.118	-1.637	0.140
			$F_{3,8}$	1.024
			adj R^2	0.006
			p	0.430
LF Index				
Coefficient	**Estimate**	**SE**	**t**	**p**
Intercept	1.758	0.265	6.647	0.002
13	-0.130	0.187	-0.693	0.508
16	-0.723	0.187	-3.620	0.005
Trial	-0.543	0.153	-3.560	0.007
			$F_{3,8}$	9.877
			adj R^2	0.710
			p	<0.001

Table 3.2 Model output from linear mixed effects models describing allometric (length-weight) relationship for *N. californiensis* before and after temperature trial 2. V.C: Variance components, AIC: Akaike's Information Criterion, BIC; Bayesian Information Criterion. $P < 0.05$ indicates statistical significance of the **time** parameter, which does not appear in the reduce model.

Coefficient	Reduced	Full
N	239	239
Fixed effects		
Intercept	-2.552(0.129)	-2.464 (0.105)
CL (mm)	0.298 (0.012)	0.298 (0.009)
Time (out)	-	-0.211(0.019)
Random Effects	V.C.	V.C.
Tank		
intercept	0.0025	0.001
Residual	0.031	0.02
AIC	-140.07	-237.41
BIC	-126.16	-220.03
p		<0.0001

Table 3.3 Table of sediment characteristics for indoor mesocosms from temperature trials. % OM; Percent organic matter, Chl *a*: chlorophyll a, Phaeo; phaeopigments. There were no statistical differences among treatments for each sample type.

Treatment	% OM	Chl *a* (mg g^{-1})	Phaeo (mg g $^{-1}$)
10	0.71 (0.34)	0.49 (0.74)	2.33 (1.04)
13	0.59 (0.05)	0.03 (0.20)	2.81 (1.14)
16	0.65 (0.06)	0.73 (0.58)	3.12 (0.42)

Table 3.4 Table showing series of models tested to describe change in carapace length (Δ CL) and LF Index for *N. californiensis* collected from field enclosures. Best fitting models are highlighted in **bold.** TDD; temperature-degree days, days; calendar days elapsed, df; degrees of freedom, AIC: Akaike's Information Criterion, BIC; Bayesian Information Criterion. * denotes statistically significant parameters.

N. californiensis					
Size Model	**df**	**adj R^2**	***p***	**AIC**	**BIC**
days* + tube + sex + site*	4	0.37	<0.0001	457.8	475.7
TDD* + tube + sex + site*	4	0.36	<0.0001	458.3	476.2
days* + tube + site*	3	0.37	<0.0001	455.5	470.9
TDD* + tube + site*	3	0.37	<0.0001	456.5	471.4
days* + site*	2	0.48	<0.0001	282.7	293.2
TDD* + site*	2	0.48	<0.0001	282.2	292.7
days* x site*	3	0.49	<0.0001	280.4	293.5
TDD* x site*	**3**	**0.51**	**<0.0001**	**278.2**	**291.2**
LF Model	**df**	**adj R^2**	***p***	**AIC**	**BIC**
days* + tube + sex + site*	4	0.34	<0.0001	46.7	66.4
TDD* + tube + sex + site	4	0.33	<0.0001	47.8	67.5
days* + tube + site*	3	0.33	<0.0001	46.2	60.2
TDD* + tube + site	3	0.32	<0.0001	47.4	61.4
days* + site*	**2**	**0.38**	**<0.0001**	**21.7**	**31.6**
TDD* + site	2	0.37	<0.0001	22.7	32.6
days* x site	3	0.40	<0.0001	19.7	32.1
TDD* x site	3	0.38	<0.0001	22.6	34.9
days*	1	0.35	<0.0001	24.2	31.6
TDD*	**1**	**0.36**	**<0.0001**	**23.6**	**31.0**

Table 3.5 Parameter values and model output from best fitting multiple regression models describing growth and lipofuscin accumulation rate in *N. californiensis* and *U. pugettensis*. TDD; temperature-degree days, CL; carapace length

N. californiensis

Carapace Length

Coefficient	Estimate	SE	t	*p*
Intercept	2.9600	0.4120	7.180	0.0001
TDD	0.0007	0.0002	3.030	0.0013
site (SB)	0.1443	0.5570	0.259	0.7960
TDD x site	0.0007	0.0003	2.452	0.0160

log(LF Index)

Coefficient	Estimate	SE	t	*p*
Intercept	-0.0551	0.1740	-0.2960	0.7679
days	0.0012	0.0002	6.903	<0.0001
site (SB)	-0.1226	0.058	-2.11	0.038

U. pugettensis

CL-Days model

Coefficient	Estimate	SE	t	p
Intercept	12.66	0.64563	20.327	<0.0001
days	0.02	0.00143	12.533	<0.0001
cohort2	-10.38	1.6246	-6.391	<0.0001
cohort3	-30.60	5.24629	-5.834	<0.0001
cohort4	-20.71	1.15595	-17.92	<0.0001
days X cohort2	0.01	0.00252	2.139	0.0385
days X cohort3	0.02	0.00632	3.352	0.0017

CL-TDD model

Coefficient	Estimate	SE	t	p
Intercept	11.780	0.755	15.611	<0.0001
TDD	0.013	0.00109	12.233	<0.0001
cohort2	-10.920	1.811	-6.03	<0.0001
cohort3	-19.057	3.723	-5.119	<0.0001
cohort4	-21.377	1.213	-17.63	<0.0001
TDD X cohort2	0.004	0.0019	2.113	0.0408
TDD X cohort3	0.005	0.00318	1.694	0.0978

LF Index model

Coefficient	Estimate	SE	t	p
Intercept	5.920	0.324	18.260	<0.0001
932 days	-2.000	0.455	-4.392	<0.0001

Table 3.6 Table of sediment characteristics measured from *N. californiensis* field sites in Yaquina Bay. IF; Idaho flats: SB; Sally's Bend, TI; Tongue Island, % OM; Percent organic matter, Chl *a*: chlorophyll a, Phaeo; phaeopigments. SD; standard deviation

Sample Type	Site	N	Chl *a*(SD)	Phaeo (SD)	% OM
inside	IF	27	6.69 (1.45)	5.44 (1.93)	0.91 (0.14)
outside	IF	27	7.41 (2.32)	4.78 (2.22)	0.74 (0.10)
inside without	IF	10	13.13 (11.72)	9.65 (4.68)	1.20 (0.23)
inside	SB	10	11.91 (2.37)	12.69 (2.14)	2.82 (0.43)
outside	SB	12	12.97 (2.45)	16.57 (6.05)	2.89 (0.60)
outside	TI	6	0.57 (0.25)	7.77 (3.90)	3.49 (0.34)

Table 3.7 Table showing series of models tested to describe change in carapace length (Δ CL) and LF Index for *U. pugettensis* collected from field enclosures. Best fitting models are highlighted in **bold.** TDD; temperature-degree days, days; calendar days elapsed, df; degrees of freedom, AIC: Akaike's Information Criterion, BIC; Bayesian Information Criterion. * denotes statistically significant parameters.

Size Model

Model	df	R^2	p	AIC	BIC
TDD +bucket +sex +cohort*	17	0.865	<0.0000	887.503	917.28
TDD* + sex + cohort*	6	0.91	<0.0001	191.34	206.32
TDD *+ cohort*	4	0.905	<0.0001	191.89	203.11
days* + cohort*	4	0.8913	<0.0001	198.52	209.75
TDD * x cohort*	**6**	**0.914**	**<0.0001**	**189.17**	**204.15**
Days* x cohort*	**6**	**0.915**	**<0.0001**	**188.23**	**203.21**

LF Model

Model	df	R^2	p	AIC	BIC
TDD + sex + date + cohort	5	0.364	0.0019	123.05	134.14
TDD + date + cohort	4	0.359	0.0011	122.53	132.03
date* + cohort	3	0.375	0.0004	120.77	128.69
date x cohort	**4**	**0.460**	**<0.0001**	**116.38**	**125.88**
date*	1	0.356	0.0002	120.03	124.78

CHAPTER 4

APPLICATION OF THE EXTRACTABLE LIPOFUSCIN AGING METHOD TO ESTIMATE MORTALITY AND POPULATION DYNAMICS OF THE BURROWING SHRIMP, *NEOTRYPAEA CALIFORNIENSIS*

ABSTRACT

Structured population models are among the most widely applied models in population ecology and typically assume that individuals can be divided into discrete classes based on stage, size or age. The lack of robust aging methods in crustaceans has caused researchers to classify individuals based on size, but differences in growth among individuals can bias population parameter estimates. Recent advances in aging crustaceans with the biochemically-produced aging pigment, lipofuscin, has created the opportunity to apply age-structured models to understand the population ecology of marine crustaceans. This study sought to apply the lipofuscin aging method to estimate mortality rate in *Neotrypaea californiensis*, a burrowing shrimp that inhabits estuaries along the US west coast. While the species is an important member of the estuarine community, *N. californiensis* also has a negative impact on oyster production in the region. As a result, managers are interested in understanding more about the population dynamics of the species and a theoretical cohort-based model was developed to explore these dynamics. Randomized surveys were conducted over a four-year period from 2011-2014 to estimate population abundance, average density and population age structure. Mortality rate was estimated to be 0.719 yr^{-1} (95% CI; $0.633\text{-}0.793 \text{ yr}^{-1}$) and did not vary significantly across cohorts. The spatial extent of the survey revealed spatial patterns in shrimp density that could be explained by variation in mortality and recruitment rates. This is the first study to apply lipofuscin aging to estimate population parameters of a thalassinidean shrimp and the methods presented here can be used to inform managers seeking to incorporate population ecology into management plans for *N. californiensis* and could potentially apply to other crustacean species worldwide.

INTRODUCTION

Structured population models are among the most widely applied models in population ecology. Introduced by P.H. Leslie in 1945, this class of model typically assumes that individuals can be lumped into discrete classes (i.e., age or stage) and that time can be divided into discrete intervals. The inability to accurately determine age in crustaceans due to their lack of hard bony structures has posed a major problem for researchers and managers attempting to apply age-structured models to estimate vital rates of ecologically or economically important species. Most population models for crustaceans have been length-based or stage-based models where size-transition probabilities are estimated from laboratory experiments or tag-and-release studies (Feinberg et al. 2006, Nilssen & Sundet 2006, Chang et al. 2012, Ohman 2012, Punt et al. 2014). However, growth rates among individuals vary greatly depending on environmental conditions (Oh & Hartnoll 2000, Hartnoll 2001, Stoner et al. 2013, Chapter 3) which can result in overlap in size classes within and among cohorts breaking down the relationship between time and size. Recent studies validating the biochemically-based lipofuscin aging method have shown promise for its use as an alternative to traditional size-based metrics (Vila et al. 2000, Bluhm & Brey 2001, Kodama et al. 2006, Puckett et al. 2008, Allain et al. 2011). The ability to estimate individual age methods creates the opportunity to apply classic age-structured models to investigate crustacean population dynamics.

The burrowing shrimp, *Neotrypaea californiensis,* inhabits soft intertidal sediments in estuaries along the US Pacific Northwest coast. Burrowing shrimps are an important component of the estuarine benthic community and play a role in estuarine ecosystem resiliency (Berkenbusch & Rowden 2003, Berkenbusch & Rowden 2006, DeWitt et al. 2004, Berkenbusch et al. 2007, D'Andrea & Dewitt 2009). Pacific Northwest estuaries also support a multi-million dollar commercial shellfish aquaculture industry primarily through the production of oysters and clams (USDA Census of Aquaculture 2013). Oysters (mostly *Crassostrea gigas*) are often grown using "on-

bottom" culture practices where the oysters are placed directly on the sediment surface of intertidal mudflats to grow (Dumbauld et al. 2006, Feldman et al. 2000). These methods subject newly planted oysters, known as "seed", to the threat of burial through bioturbation by *N. californiensis* which can result in significant economic losses to growers (Feldman et al. 2000). The presence of burrowing shrimp pests on oysters beds are a concern to growers that operate on grounds overlapping with burrowing shrimp populations (Feldman et al. 2000, Chew 2002, Doughton 2015) and for over 50 years the aquaculture industry had applied topical pesticides to intertidal mudflats to control shrimp populations in oyster beds (WDFW 1970, Feldman et al. 2000). After increasing environmental concerns over the impacts associated with application of the pesticide (WDFW and WDOE 1992), the industry agreed to transition to an integrated pest management plan (IPM) in an attempt to improve control while minimizing environmental impacts (Dumbauld et al. 2006).

Successful implementation of an IPM plan requires methods to accurately assess burrowing shrimp populations and the development of population models (DeWitt et al 1997, Dumbauld et al. 2006, Bosley & Dumbauld 2011). Several studies have examined life-history aspects of *N. californiensis*, describing details on growth, fecundity and population age structure which could be used in developing a population model (Bird 1982, Dumbauld et al. 1996, Bosley & Dumbauld 2011). These studies indicated growth rate varied spatially among populations and using the biochemically based lipofuscin-based aging method, Bosley & Dumbauld (2011) showed that growth can vary significantly even within a cohort at a given location. Bosley & Dumbauld (2011) also demonstrated that *N. californiensis* might have a lifespan up to 13 years, more than twice the previous estimate. In light of the new information regarding age and growth in *N. californiensis*, it is clear that estimating population parameters from size-frequency data would produce inaccurate parameter estimates that may not reflect the true population dynamics of the species.

Lipofuscin-based aging methods overcome the challenges associated with traditional sized-based methods of age determination for *N. californiensis* like spatial and temporal growth variability (Chapter 2). To date, most lipofuscin studies have involved validation of the methodology (Vila et al. 2000, Bluhm & Brey 2001, Kodama et al. 2006, Puckett et al. 2008, Allain et al. 2011) and a few have used the methods for conducting a demographic assessment (Sheehy & Ettershank 1988, Bluhm et al. 2001, Ju et al. 2001, Harvey et al. 2010, Bosley & Dumbauld 2011). While these studies have provided details regarding growth and life-history for several crustacean species, none have extended application of the method to the estimation of population vital rates or development of a population dynamics model which could be used as part of a management framework.

The ability to predict population level changes has become the basis for management of biological resources and fisheries and wildlife conservation (Hilborn & Walters 1992, Udevitz & Ballachey 1998, Beissinger & McCullough 2002, Morris & Doak 2002). Population abundance can be described by the balance between recruitment and mortality rates (Caswell 2001) and understanding these rates allows for prediction of future populations. Since burrowing shrimp have pelagic larvae that disperse in the coastal ocean, recruitment of *N. californiensis* is likely linked to the oceanic environment and may be less predictable (McCrow 1972, Dumbauld et al. 1996, Tamaki et al. 2010, Dumbauld et al unpublished). The sedentary life-style of the shrimp, however, allows the adult population to be easily sampled. With the availability of robust aging methods, application of age-based models can be applied to estimate population parameters for *N. californiensis*.

The goal of our study was to apply the lipofuscin aging method to estimate natural mortality rate for *N. californiensis* populations in Yaquina Bay, Oregon, with a cohort-based approach. The objectives were to 1) conduct annual population assessments of *N. californiensis in* Yaquina Bay, Oregon 2) apply lipofuscin-based aging methods to determine age structure and estimate age-specific mortality rate for the measured

population and 3) construct a cohort-based simulation model that can be used to predict changes in Yaquina Bay shrimp populations under different recruitment and mortality scenarios relevant to their ecology and management. This study represents the first application of lipofuscin aging to understand population dynamics of a burrowing shrimp. In addition, the methodology developed in the study can be used to estimate mortalities for *N. californiensis* populations in other estuaries and provide a tool to be used in in development of successful management plans for burrowing shrimp in the Pacific Northwest and potentially for other crustaceans worldwide.

MATERIALS & METHODS

Study site

Population parameters were estimated for a subset of the total population of *N. californiensis* located on the Idaho flats mudflat in Yaquina Bay, Oregon, USA (44°37'8.40"N, 124° 2'27.29"W) located on the central Oregon coast (Figure 4.1). Population densities can reach up to 500 shrimp m^{-2} in some areas of the bay (DeWitt et al. 2004) and the shrimp can be found at tidal elevations ranging from approximately -2.0 meters relative to mean lower low water (MLLW) to about +3 MLLW (D'Andrea unpublished, Dumbauld et al, in revision). We sampled an established population of *N. californiensis* located in the upper intertidal (+2-3 MLLW) that represents about half of the total population on Idaho flats in Yaquina Bay, OR. The survey encompassed a range of densities and was concentrated on a subset of the total population allowing greater sampling effort to be made to achieve more precise population parameter estimates.

Sample collection

Total abundance of *N. californiensis* in the area we sampled was estimated from annual population surveys conducted from 2011 to 2014. Spatial extent of the population each year was determined by using population bed edges defined by walking along the population edge with a high precision Trimble GeoXT GPS. In areas where a clear bed

edge was not visible bed edges were defined by walking within a zone where burrows were clearly increasing in density to one side and decreasing on the other, usually within a 3-meter distance. Bed edge data were imported into Trimble GPS Pathfinder Office v. 4.20 (Trimble Inc., Sunnyvale, CA) for differential correction and exported as an ERSI shapefile. The spatial bed edge data was then converted into spatial polygons using 'rgdal' (Bivand et al. 2015) and 'raster' packages (Hijmans and van Etten 2012) in the R (R core development team, 2014). One hundred random survey sites were selected within the shrimp bed polygon in 2011. In 2012 the number of sites was increased to 150 and sampling locations were selected using the pseudo-random Generalized Random-Tesselation Stratified (GRTS) survey design (Kincaid & Olsen 2015). Cross-validation tests of model accuracy showed that population abundance estimates were not significantly improved by adding more survey points so 100 survey points were selected in 2014 (Tomczak 1998).

Population surveys involved navigation to the pre-selected locations with a Trimble GeoXT high precision GPS unit. At each site burrow counts were taken within a 0.25 m^2 quadrat. After burrow counts were determined, 10 of the survey locations were randomly selected to collect core samples (Fig 4.1). Core samples were taken to get a representative sample of the population to acquire population age structure and also determine the relationship between shrimp density and burrow count for estimating total population abundance (Dumbauld et al. 1996, Dumbauld et al. in revision). Core sample locations were selected by dividing burrow quadrat locations into 5 different strata based on burrow counts and randomly selecting 2 points within each of the 5 strata (Fig 4.1A). Core samples were taken with a 0.125 m^2 stainless steel core to 60 cm depth at each randomly selected location. The number of shrimp burrows within the core area was recorded prior to pushing the core into the substrate then material from each core was excavated, sieved with 3mm mesh and sorted for shrimp. All shrimp were sexed and measured for carapace length prior to being frozen at -80°C for lipofuscin age analysis. A minimum sample size of 200 animals is preferred for accurate age structure analysis. If a

total of 200 individuals were not collected in the first 10 core samples, randomly selected "back-up" core locations were sampled in the high density strata until the minimum sample size was reached. Sex ratios were tested for equal proportions using a chi-square proportions test.

Spatial interpolation model for shrimp abundance

Estimation of total shrimp number relied on the relationship between burrows and shrimp. This relationship was determined by conducting simple linear regression of burrow number (# m^{-2}) as a function of shrimp number (# m^{-2}) for each year of the survey. The regression model for each year was then used to convert burrow counts to shrimp numbers for estimation of total population abundance with a deterministic spatial interpolation model.

An inverse distance weighed (IDW) spatial interpolation model was used to estimate total shrimp abundance within the area sampled using the raster package in R (Hijmans & van Etten 2012). Ideal parameters for the IDW model were determined with cross-validation of IDW models (Tomczak 1998) using combinations of inverse distance weighted power (idp) values ranging from 0.5 to 3 and number of neighbors (nmax) from 2 to 18. The models were most accurate with idp = 1.5 and nmax = 6 and were used for creating IDW models for estimating shrimp abundance for all years using a 5 m^2 cell size. The interpolation model was bounded by the shrimp bed edge polygon and shrimp total estimated by summing shrimp abundance values over all cells within the polygon. Variance for total abundance estimates was determined using the blocked bootstrapping method with the 'sperrorest' package (Brenning 2012) and accounted for the spatial autocorrelation of the survey data (Lahiri et al. 1999, Lahiri & Zhu 2006, Brenning 2012).

Estimation of population age structure

Age structure was constructed with cohort analysis of lipofuscin frequency histograms. Males and females were combined to ensure a sufficient number of specimens for the cohort analysis and because lipofuscin accumulation rate is not significantly different between the sexes (Chapter 3). Lipofuscin values were determined for all shrimp greater than 6.5 mm carapace length collected in population surveys using the methods described in Chapter 3 (see 'Measurement of Lipofuscin' section previous chapter). Cohort analysis was conducted by dividing lipofuscin frequency distributions into a mixture of Gaussian probability curves, each representing an estimated age class. The analysis was completed separately with data from each year using the package 'mixdist' (MacDonald 2015) in R. The 'mixdist' algorithm works by applying maximum likelihood methods to estimate means and variance for a mixture of distributions based on preselected starting values and application of constraints on the parameters (Du 2002). The lipofuscin metric used in the analysis was a relative concentration index where the total concentration of lipofuscin in the tissue extract is normalized to the total protein with units ng lipofuscin μg^{-1} protein. The normalized index, LF Index, is used to account for variation in animal size and tissue dissection efficiency. A LF Index bin size of 0.20 ng ug^{-1} was used for the analysis because it allowed for the greatest resolution of potential modes the histogram. Initial starting values were selected visually based on where modes appeared to be present. No additional constraints were placed on parameter values and the model for each year was tested for goodness-of-fit using a chi-square statistic. Ages were assigned to modes present in frequency histograms based on mean and standard deviation for the lipofuscin accumulation rate presented in Coleman et al. (in prep; 1.523 ± 0.046 ng μg^{-1} yr^{-1}).

Estimation of shrimp mortality rate

Average annual mortality rate for *N. californiensis* in Yaquina Bay was determined using a catch curve analysis (Robson & Chapman 1961) of numbers-at-age

for cohorts collected over the four year survey. Juvenile shrimp (shrimp < 6.0 mm) were not able to be aged and therefore did not appear as part of the lipofuscin-based age structure. Total population density of adults each year was determined by subtracting the proportion of juveniles from total abundance estimates and then adult shrimp abundance was converted to numbers-at-age based on lipofuscin concentration. Mortality was estimated by determining the rate of decline in shrimp number as each cohort progressed through time by fitting a discrete-time exponential decay mortality function to numbers at age data for all cohorts combined.

$$N_{t+1} = N_t e^{-k} \tag{1}$$

Where N_t is number of animals in a cohort at time t, N_{t+1} is the number of animals alive at the beginning of time $t+1$, and k is the exponential decay coefficient. Mortality rate was determined using the linearized form of the model which allowed k to be estimated as the regression slope.

$$\log(N_{t+1}) = -k + \log(N_t) \tag{2}$$

$$S = \frac{N_{t+1}}{N_t} \tag{3}$$

$$M = 1 - S \tag{4}$$

In these equations S is the fraction of animals surviving each time step and M is the mortality rate (yr $^{-1}$). Differences in mortality across cohorts was tested with analysis of co-variance (ANCOVA) including cohort as a covariate. A sum of squares F-test was used to compare the full model which included cohort to a reduced model with only time as a predictor variable. Average mortality rate and its error estimated from the best model was then used to conduct population simulations.

Life-table population simulations

      A perturbation analysis was done to test the effects of changes in mortality and recruitment on the population dynamics of *N. californiensis*. This theoretical analysis explored the response of population density under different recruitment and mortality scenarios. The model assumed recruitment into the adult population occurred at age 2, the age at which the shrimp are captured in our survey as determined from lipofuscin-based age structure, and lifespan for *N. californiensis* was 13 (A_{max}) based on previous estimates for the longevity (Bosley & Dumbauld 2011) and therefore shrimp were assumed to exit the model after age 13.

Single cohort mortality calculations

      The effect of mortality rate on population density of a cohort was investigated by evaluating the cohort model equations over a range of mortality levels (Equation 5). Mortality rates tested were 0.9, 0.5, 0.3, and 0.1 yr^{-1}.

$$N_{t+1} = N_t\,(1-M)^t \tag{5}$$

Multi-cohort equilibrium scenarios

      Projections of the cohort model were completed to examine the outcome of different combined recruitment and mortality scenarios. Population density was modeled assuming constant annual recruitment and constant mortality in the following four scenarios using Equation 6: high recruitment / low mortality; high recruitment / high mortality; low recruitment / high mortality; low recruitment / low mortality. High and low recruitment values were 60 and 20 shrimp m^{-2} respectively. High and low morality rates were 0.8 and 0.2 yr^{-2}. Recruitment values were selected because they represent range of recruitment values that may be expected based on empirical recruitment data from Yaquina Bay, Oregon (Dumbauld unpublished)

$$N_{t+1} = R + N_t(1 - M) \tag{6}$$

For this constant model, the population will conserve on an equilibrium level (N_{eq}):

$$N_{eq} = R/M \tag{7}$$

Stochastic recruitment model

A stochastic model was constructed that allowed recruitment to vary annually. This was done by setting recruitment (entry of age 2 shrimp into the model) as a random variable with a negative binomial distribution (mean = 11 and dispersion = 0.4). These parameters were estimated by visually fitting a negative binomial distribution to age 2 density data from surveys conducted in Yaquina Bay from 2005 – 2015 (Dumbauld unpublished). The model assumed that mortality rate was constant within a cohort but was allowed to vary across cohorts by setting *k* as a normal random variable with a mean and standard deviation equal to the mortality fraction determined from ANCOVA. Projections were done with into a t_m x A_{max} matrix where t_m = the total number of time steps in the model. Numbers-at-age for each cohort was calculated with the following equation:

$$N_{t+1,a+1} = N_{t,a}e^{-X} \tag{8}$$

Where,

$$X \sim Normal(k, \sigma_k) \tag{9}$$

$N_{t,a}$ is the number of individuals in a cohort alive at age *a* at time *t* and $N_{t+1, a+1}$ is the number of individuals in a cohort surviving to the following year. Total shrimp numbers were converted to shrimp density (m^{-2}) by summing numbers-at-age for each time step and dividing by total area (*A*).

$$\text{Shrimp Density}_t = \frac{1}{A}\sum_{a=1}^{n} N_{t,a} \qquad (10)$$

The mean and variance for shrimp density at each time step was determined for the stochastic scenario with a Monte Carlo simulation where calculations were repeated 500 times. The model was projected for 15 years.

RESULTS

   The total area occupied by *N. californiensis* changed over the four years that our surveys were conducted showing a peak in 2013 with the population covering 91,596 m^{-2} (Table 4.1, Fig 4. 2). The total number of shrimp in the area sampled ranged from a high of over 7 million in 2013 to 3.9 million in 2014. Shrimp abundance increased by 34.1% from 2012 to 2013 and decreased by 14.3 % and 44.3% from 2011 to 2012 and 2013 to 2014 respectively. Despite the fluctuations in total shrimp numbers, overall shrimp density declined over the four years (~ 50%) surveyed. The decline in density was evident in spatially interpolated maps of the surveyed shrimp population which also showed expansion of the shrimp bed to the north in 2013 and in 2014 a large extent of the population to the south had disappeared (Fig 4.2). The burrow to shrimp relationship also varied slightly each year with the steepest slope in 2011; ~ 2 burrows for every shrimp (Table 4.2). In 2013 and 2014 the ratio had reduced to ~ 3 burrows per shrimp (Table 4.2, Fig 4.3).

   Carapace lengths of shrimp sampled in cores indicated a fairly stable size structure. Juveniles were clearly evident in core samples from 2011 and 2012, presumably shrimp that had recruited to the population as postlarvae the previous year (Figure 4.4). Juveniles (shrimp < 6 mm CL) made up 17.3% and 12.3% of the total population in 2011 and 2012 respectively. There were few small shrimp collected in 2013 and 2014 core samples (Table 4.3). Average adult CL was similar across years averaging around 10 mm in 2011, 2012, and 2013 but was slightly smaller on average in 2014

(mean CL = 9.78 ± 1.65; Fig 4.4). Sex ratio of adults appeared to be dominated by females (~2:1) in all years but the difference was only statistically significant in 2011 (X^2 8.741, p = 0.003; Table 4.4).

The mixture of Gaussian distributions determined with the 'mixdist' model fit the actual LF Index frequency data as determined by chi-square goodness-of-fit tests (Table 4.5), but there was a fair amount of overlap between the different curves. Evaluation of population age structure showed age structure to be stable over the four years with ages 2 to age 7 present in core samples with the exception of 2014 when age 6 shrimp were absent in the core samples. In all years, age 4 animals were the dominant year class (Table 4.5, Fig 4.5). This was likely a result of variable growth rates causing only a fraction of age 2 and 3 animals being large enough for LF analysis (< 6.5 mm CL). By age 4 all animals had grown to a size where they were captured with our sampling methods and where LF analysis could be done, therefore the mortality model only included numbers-at-age data for shrimp age 4 and older (Table 4.6, Fig 4.6). Mortality rate, estimated with Equations 2-4, was determined to be 0.757 yr^{-1} (95% CI; 0.613-0.858 yr^{-1}, Table 4.7) after controlling for variation in cohort. Comparison of the full model which contained cohort as a covariate vs a reduced model indicated that cohort was not a significant predictor of mortality rate for *N. californiensis* on Idaho Flats (Fig 4.7, Table 4.7). Mortality rate estimated from the reduced model was 0.719 yr^{-1} (95% CI; 0.633-0.793 yr^{-1}).

Theoretical life-table projections showed that a single cohort experiencing the mortality rate of 0.719 yr^{-1} would be reduced to very low population densities after 4 years (0.6% of original abundance) regardless of recruitment strength (Fig 4.8A). Conversely, changes in mortality rate had a pronounced effect on the density of a cohort over time (Fig 4.8A). The effect of mortality rate on change in density was non-linear, with smaller changes in mortality rates having a greater effect on density when mortality rates were low. The combined scenarios assumed that annual recruitment mortality rates were constant and allowed prediction of change in population density as the population

reached equilibrium with multiple overlapping generations (Fig 4.8B). The low mortality and high recruitment example showed that following colonization a population can achieve a high density approaching equilibrium in 20 years (Table C1 in the appendix). The low recruitment example showed population densities increasing slowly to a much lower equilibrium density (Fig 4.8B). In both high mortality scenarios, the population reached equilibrium quickly (4 years) and were maintained at overall low densities (Fig 4.8B, Table C1).

The stochastic model assumed recruitment of age 2 shrimp occurred at low levels with periodic large events (Figs 4.9A & B), which is reflective of actual measurements from population surveys in Yaquina Bay. As cohorts progressed through the model, population density tended to decrease until the next major recruitment event occurred. The average density estimated from 500 iterations of the stochastic model was 14.98 shrimp m^{-2} (Fig 4.9C) which suggests that a small population can persist with generally low and infrequent recruitment.

DISCUSSION

The ability to accurately age crustaceans has hindered the application of standard age-structured models for estimating vital rates (Hartnoll 2001, Bosley & Dumbauld 2011, Punt et al. 2014). Recent developments of alternative aging techniques (O'Donovan & Tully 1996, Ju et al. 2001, Puckett et al. 2008, Harvey et al. 2010) and improved methods of population assessment have created the opportunity to understand crustacean population dynamics in a way that was not previously possible. The primary goal of this study was to apply modern techniques to estimate average annual mortality rate for a population of *N. californiensis* in Yaquina Bay, Oregon, and determine if that rate was constant over time. The cohort-based approach showed a non-linear exponential decay function described burrowing shrimp mortality rate in the area we sampled and this function was not statistically different among cohorts. Our data agrees with work by others confirming thalassinidean shrimp are characterized by relatively high mortality

rates. Mortality estimated from lipofuscin-based measurements of age was consistent with similar estimates of mortality determined from size class analysis of *N. californiensis* and other thalassinidean shrimps. Feldman et al (2000) tracked cohorts of *N. californiensis* over two years and estimated mortality rates to be 0.75 yr^{-1} and 0.22 yr^{-1} for males and females, respectively, but had difficulty in estimating the parameter when females immigrated from outside the sample area. Conides et al. (2012) found natural mortality rate of the mud prawn, *Upogebia pusilla*, to be 0.93 yr^{-1} and Pezzuto (1998) calculated annual mortality rates of 0.78 yr^{-1} and 0.71 yr^{-1} for male and female *Neocallichirus mirim*.

Accuracy in estimating population parameters with lipofuscin

The estimation errors associated with the parameters (proportion and mean LF Index-at-age) acquired from the 'mixdist' algorithm were quite large in some years. Even though the overall model for mixture of frequency distributions fit well, the high level of uncertainty in the parameter estimates suggests that they are not highly robust. The modal progression analysis we conducted uses a normal approximation to describe the distribution of lipofuscin concentration within a cohort. While application of the Gaussian distribution is most common for conducting age-structure analysis, lipofuscin concentration frequency data may be best described by a different probability distribution, such as a Gamma distribution, and further work should be done to refine cohort analysis as it applies to lipofuscin data. Uncertainty in the parameter estimates may have resulted from fitting the model with limited constraints on the estimates, but could also be explained by significant overlap of lipofuscin concentration distributions among cohorts.

The primary advantage of using analysis of lipofuscin to estimate crustacean age is that accumulation rate may be consistent over broad temporal and spatial ranges and can provide an index of age, despite clear differences in growth. Previous work has shown *N. californiensis* growth rates are variable and in some cases shrimp can shrink

when environmental conditions are poor which has led to a decoupling of the age-size relationship both within and across populations (Chapter 2, Coleman et al., unpublished data, Bosley & Dumbauld 2011). Even though growth rates are variable, lipofuscin accumulation is fairly consistent across populations and across cohorts. Coleman et al (personal communication) estimated lipofuscin accumulation rate in multiple cohorts grown in outdoor mesocosms and found 91% of the variability in LF Index value was explained by time whereas body size was not correlated. Our data showed significant overlap in LF index frequency histograms for each survey year. Because, formation of lipofuscin is controlled by metabolic processes, the rate in which it accumulates is subject to considerable individual variability and has been linked to environmental conditions (i.e., temperature) under natural field conditions (Chapter 3, Sheehy et al. 1995, O'Donovan & Tully 1996). Laboratory and field experiments have found temperature to have a weak effect on lipofuscin accumulation in *N. californiensis* but other factors that influence metabolic rates such as genetic background, oxidative stress, and salinity may be important in dictating lipofuscin accumulation (Chapter 3, Terman 2001, Allan et al. 2006, Hiebenthal et al. 2012). Timing of recruitment may also explain, in part, overlapping modes in lipofuscin concentration frequency histograms. Settlement of *N. californiensis* typically occurs between July and October and has been shown to occur as late as January or as early as March depending on the year and the location (Dumbauld et al. 1996, Dumbauld unpublished). Long recruitment periods would increase variability in mean LF Index-at-age estimates, broadening modes and increasing overlap in frequency data. Despite these challenges in estimating "true" age for *N. californiensis* using lipofuscin concentration, the method has proven to be a more accurate metric of age than body size and allows for comparison across and within populations where growth rates can vary significantly.

Patchiness in shrimp distribution

Biological populations are primarily governed by two sets of opposing processes; recruitment and mortality; immigration and emigration. For sedentary benthic species like *N. californiensis*, immigration and emigration are generally ignored because they exhibit little or no movement (Rosenberg 1974, Thrush 1991, Hewitt et al. 1997, Castorani et al. 2014). Therefore, observed patterns of abundance for benthic macrofauna are usually described by the opposing forces of recruitment and mortality (Olaffsson 1994). Because of their limited motility, these processes likely act on populations at a fine scale and result in a mosaic of densities across the landscape (Thrush 1991, Hewitt et al. 1997, Lundquist et al. 2010). The burrow count densities we encountered ranged from 0 to > 200 burrows m^{-2} and exhibited clear spatial structure showing "hotspots" of high density in localized regions. Patchy distributions of benthic species have been studied for several decades with most work being focused on settlement and subsequent recruitment as the primary process controlling populations (Underwood & Fairweather 1989, Morrisey et al. 1992, Fraschetti et al. 2002). Long-term settlement records for *N. californiensis* in Yaquina Bay have shown recruitment patterns to be sporadic with relatively low shrimp numbers recorded each year on average, yet adult populations persist in high densities in some areas (Figs 4.2 & 4.9A). Population projections of the cohort model show that high population density can be explained by either increased survival, increased recruitment or both.

To date, much of the population data collected on *N. californiensis* has been from high density locations (Bird 1982, Dumbauld et al. 1996, Feldman et al. 2000, Bosley & Dumbauld 2011). Bosley and Dumbauld (2011) used lipofuscin-based aging methods to estimate age structure of *N. californiensis* in a high density patch and found 13 age classes present in their samples. Our projections showed shrimp density to be relatively sensitive to changes in mortality. Using the mortality equations, a cohort experiencing a low mortality rate of 0.1 % yr $^{-1}$ would have only been reduced to 34.7 % of its original size after 10 years vs < 0.001 % in a cohort of the same size under the high mortality

scenario (M = 0.72 % yr^{-1}). These conditions would broaden the age structure with the inclusion of a higher proportion of older shrimp in the population and increase the estimated survival rate based on age-structure data (Udevitz & Ballachey 1998, Carey et al. 2008, Udevitz & Gogan 2012). The survey we conducted was designed to capture as much variability in age distribution and mortality as possible throughout the area that we surveyed and identify spatial changes to the overall population that would not have been observed if only a single location had been sampled. Our survey observed only 7 age classes as determined with lipofuscin aging but sampling took place over a larger spatial extent and covered areas of low population density where mortality is likely to be much higher, especially along bed edges where environmental stressors or greater exposure to predators may limit populations (Bertness et al. 1985).

The role of post-larval dispersal in regulating population dynamics in soft-bottom systems has begun to emerge as a phenomenon responsible for flux of animals in and out of patches (Olaffsson 1994, Valanko et al. 2010, Pacheco et al. 2013). Post-settlement juveniles or adults can be triggered to actively disperse by abiotic or biotic cues (Lenihan & Micheli 2001, Commito et al. 2005, Kumagai 2006). In the case of *N. californiensis*, new settlers are typically sampled within the dense shrimp beds along with the established adult population and are often found in low abundance. This pattern is consistent with other studies that have shown dense aggregation of adults inhibit settlement and recruitment of juveniles through direct feeding or disruption of sediments (Woodin 1976, Bertness et al. 1985, Olaffsson 1994). Our multi-year survey showed an expansion of the shrimp population that was likely a result of a settlement event in 2010. However, densities in the newly colonized area were initially low and declined following colonization. With a mortality rate of 0.719 yr^{-1}, reduction in abundance was likely a result of mortality but it may have also involved some migration of adults to replenish loss within a nearby high density patch. Some horizontal movement of adult *N. californiensis* has been documented (Peterson 1984, Posey 1986, Castorani et al. 2014) and there are numerous anecdotal reports by oyster growers and bait harvesters that

shrimp move in the water column (Dumbauld 1994). Low density areas or bed edges may provide a refugia for juveniles to settle and grow before recruiting to the adult population. This "edge effect" has been observed in other benthic invertebrates and may be an important process in population regulation (Tamaki & Ingole 1993, Minchinton 1997, Whitlatch et al. 1998). Further investigation into the movement of adult and sub-adult shrimp within shrimp aggregations would confirm whether immigration is a factor to consider in describing the population dynamics of *N. californiensis.* In addition, a greater understanding of the settlement process and the primary causes of mortality following settlement would provide insight into how shrimp populations persist in a heterogeneous estuarine environment.

Predicting population change with cohort-based model

Like many benthic species with prolonged larval periods, *N. californiensis* populations are "open" in that larvae are flushed from estuaries into the nearshore coastal ocean and therefore recruits can come from outside populations (Fairweather 1988, Underwood & Fairweather 1989, Grosberg & Levitan 1992, Caley et al. 1996) and some researchers have been successful in linking recruitment strength of marine invertebrates to climate variables (Caley et al. 1996, Shanks & Roegner 2007, Menge et al. 2009, 2011, Woodson et al. 2012). No studies to date have been able to do the same for predicting settlement in *N. californiensis.* We constructed of a cohort model which allowed for a theoretical investigation of population level changes resulting from different recruitment and mortality scenarios. Recruitment in our model was defined as animals that enter a population at age 2, the age that they may be captured by a 3mm mesh sieve. The model makes no assumptions about how the animals arrived, whether through immigration or settlement, but also assumes that the animal will not migrate out of the population following recruitment. It was assumed that projected changes in cohort density are a result of mortality only. While these assumptions may be simplistic, the application of a

simple model with minimal data requirements can provide a useful tool in the development of management frameworks.

As the oyster industry in the Pacific Northwest has attempted to move towards integrated pest management for these shrimp, the need to understand how populations change on oyster grounds following a settlement event has been a primary focus of ecological research on *N. californiensis* (Dumbauld et al. 1996, 2006, Feldman et al. 2000, Bosley & Dumbauld 2011). Based on empirical estimates of mortality, our model showed that a cohort will experience rapid decay following a colonization event. This result suggests that a population may be reduced to levels below which they can have a significant negative impact on oysters a few years following settlement (Feldman et al. 2000), even under a high recruitment scenario. The single cohort example is somewhat unrealistic however, because large settlement events for *N. californiensis* occur consecutively and/or periodically (Dumbauld et al. 2006, Dumbauld unpublished). In species with multiple overlapping generations, regular settlement causes populations to increase in size as the year classes are effectively stored in the population (Cole 1954, Chesson 1983, Caswell 2001). This is what we and others have observed in *N. californiensis* with stable size and age distributions (Dumbauld et al. 1996, Feldman et al. 2000, Bosley & Dumbauld 2011) and has been seen in other similar burrowing shrimp species (Tamaki et al. 1997). Consequently, prolonged periods of repeated recruitment can have a major influence on shrimp populations. We attempted to determine the effect of variable recruitment on shrimp density by creating a stochastic recruitment model which took into account the irregular nature of settlement for *N. californiensis*. The model results showed that *N. californiensis* can persist at an average low abundance (~ 14 shrimp m^{-2}) with generally low, irregular recruitment and high mortality. The stochastic model also showed shrimp densities to increase then steadily decrease much like has been observed in previous and current monitoring programs of *N. californiensis* populations (Bird 1982, Dumbauld et al. 1996, Feldman et al. 2000, Dumbauld et al. in revision).

The persistence of burrowing shrimp populations is clearly linked to the rate at which new animals enter the population and the rate at which they die. Lipofuscin aging has provided evidence that *N. californiensis* is a long-lived animal which explains why populations are stable despite unpredictable recruitment (Chesson & Warner 1981, Chesson 1983). However, our model projections showed temporal patterns of recruitment had a marked effect on overall population density. Settlement strength is a very difficult process to quantify and in many cases settlement does not correlate to recruitment in benthic invertebrates (Fraschetti et al. 2002, Pineda et al. 2010). Assuming that mortality remains constant after juveniles recruit into the adult shrimp population, simple calculations can be used to estimate the abundance of the following year's shrimp population within areas of interest using data on recruitment and population abundance from the current year. These predictions will be useful in guiding management decisions regarding when to control shrimp and where control may be needed, if at all. Future work should consider development of survey methods to quantify the spatial and temporal distribution of juveniles within and around areas of interest and also determination of a "threshold density" where shrimp have a significant impact on shellfish production.

This study is the first to apply lipofuscin-based aging to estimate population parameters and inform population models for a thalassinidean shrimp. We also identified several areas of research that would provide information to further refine our understanding of burrowing shrimp population ecology including, identifying the major causes of adult mortality and quantification of the modes and rates of migration. The survey methods and cohort model developed in this study can be used to improve assessments for *N. californiensis* populations in estuaries along the US west coast and be incorporated into decision making frameworks to provide guidance on where and when to implement management practices if needed.

ACKNOWLEDGMENTS

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Figure 4.1 A) Map of Yaquina Bay, Oregon showing location of Idaho Flats where *N. californiensis* was sampled. Dark grey represents land, light grey is intertidal flats and white is water channel. B) Shrimp population polygon (tan) from 2011 with sampling grid of 2011 survey overlaid. Crosses indicate quadrat sample sites and colored circles showing stratified core sampling sites. Grey represents land/marsh, white is intertidal flats.
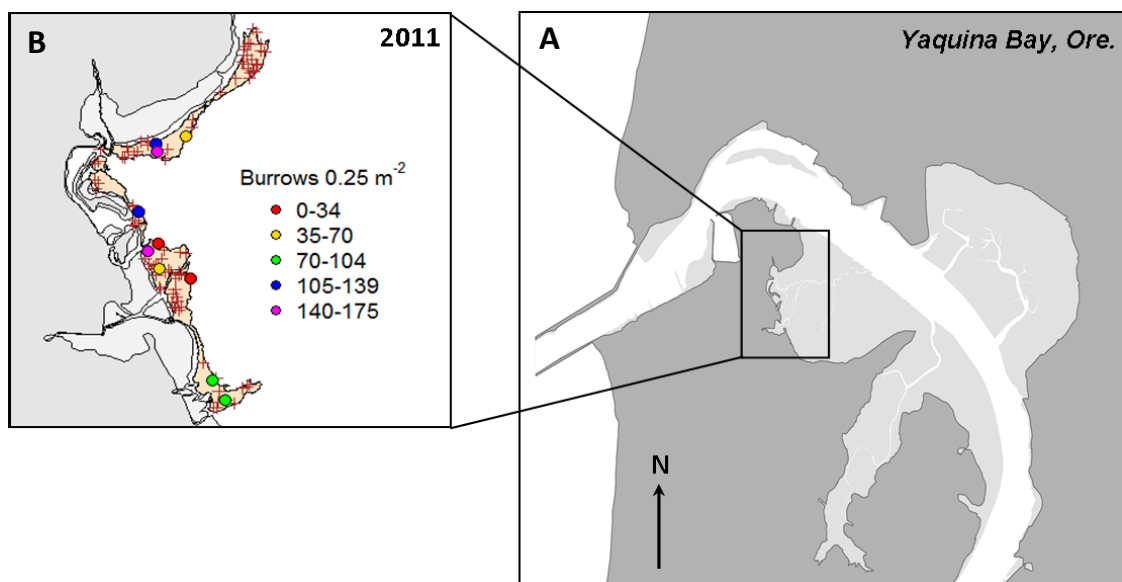
Figure 4.2 Inverse distance weighted interpolation maps of *N. californiensis* population density in Yaquina Bay for years 2011 – 2014 from Idaho Flats in Yaquina Bay. Warm colors indicate high shrimp densities. White area is land and grey represents intertidal flats. Plotting area is the same as shown in Fig 4.1B.
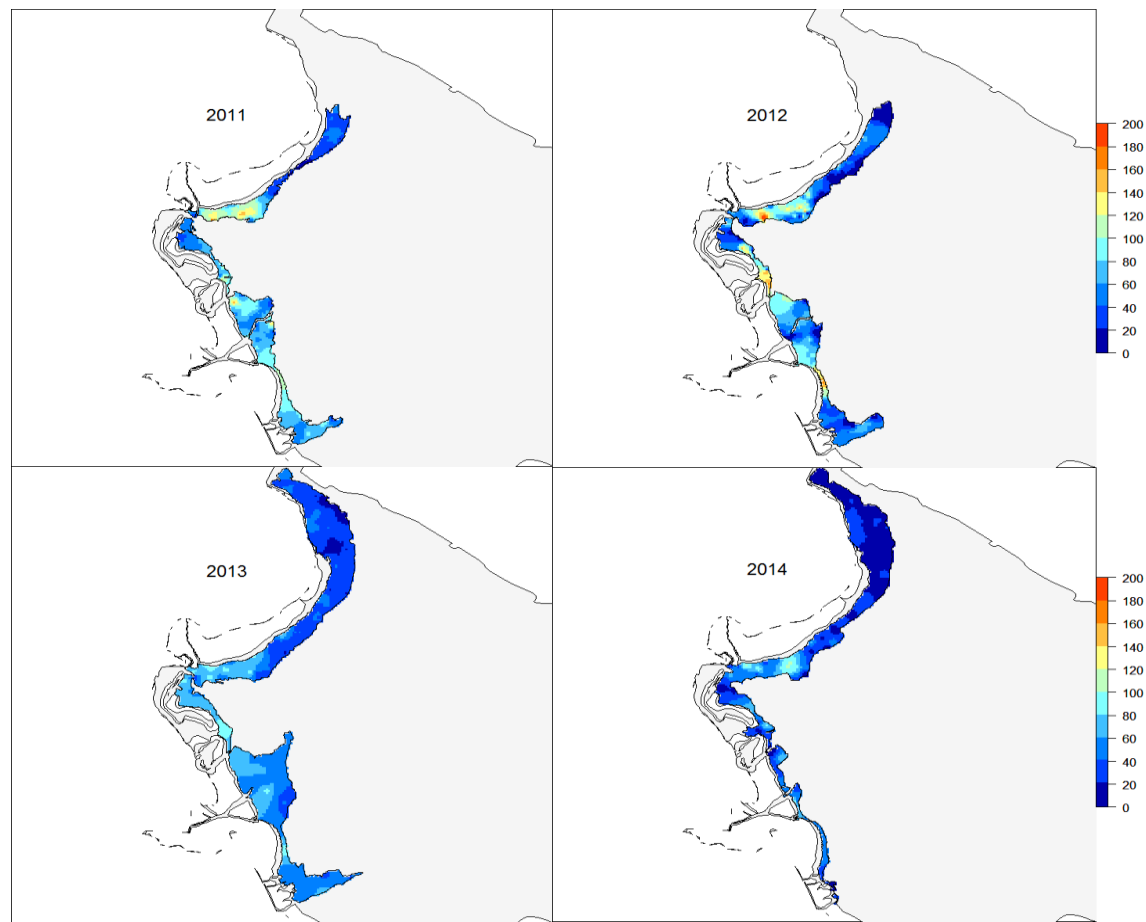
Figure 4.3 Plot showing the burrow to shrimp relationship with linear regressions used to estimate shrimp abundances from burrow counts in each survey year. Regression coefficients are found in table 4.2.
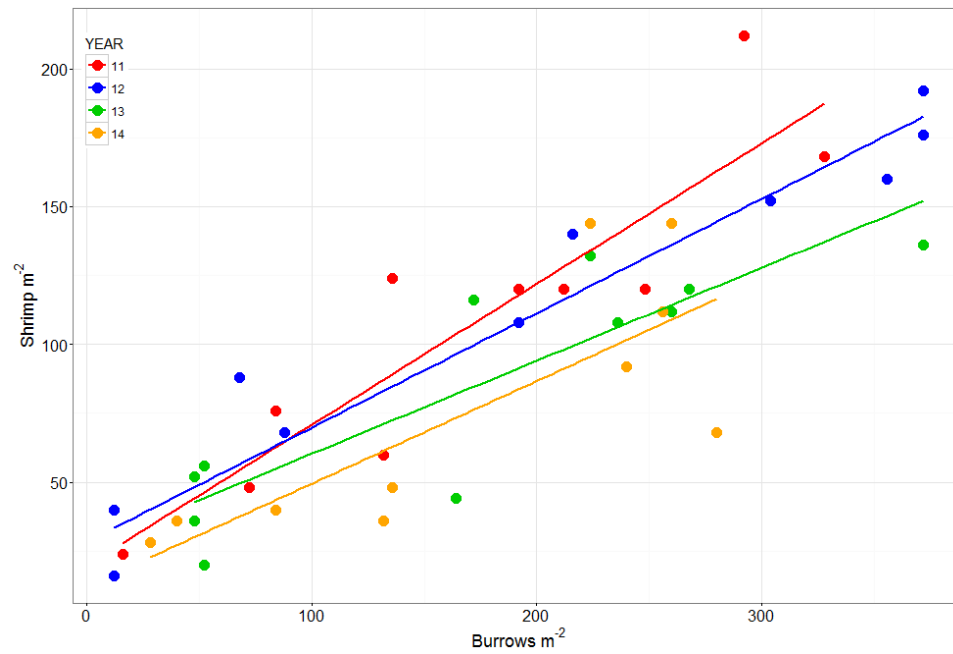
Figure 4.4 Carapace length frequency distributions of *N. californiensis* collected from annual population surveys on Idaho Flats. Bars are divided by maturity and sex. Mean carapace length (mm) for each year is shown in upper right corner of each panel. Juveniles are animals < 6.5 mm CL. These shrimp were not aged using lipofuscin.
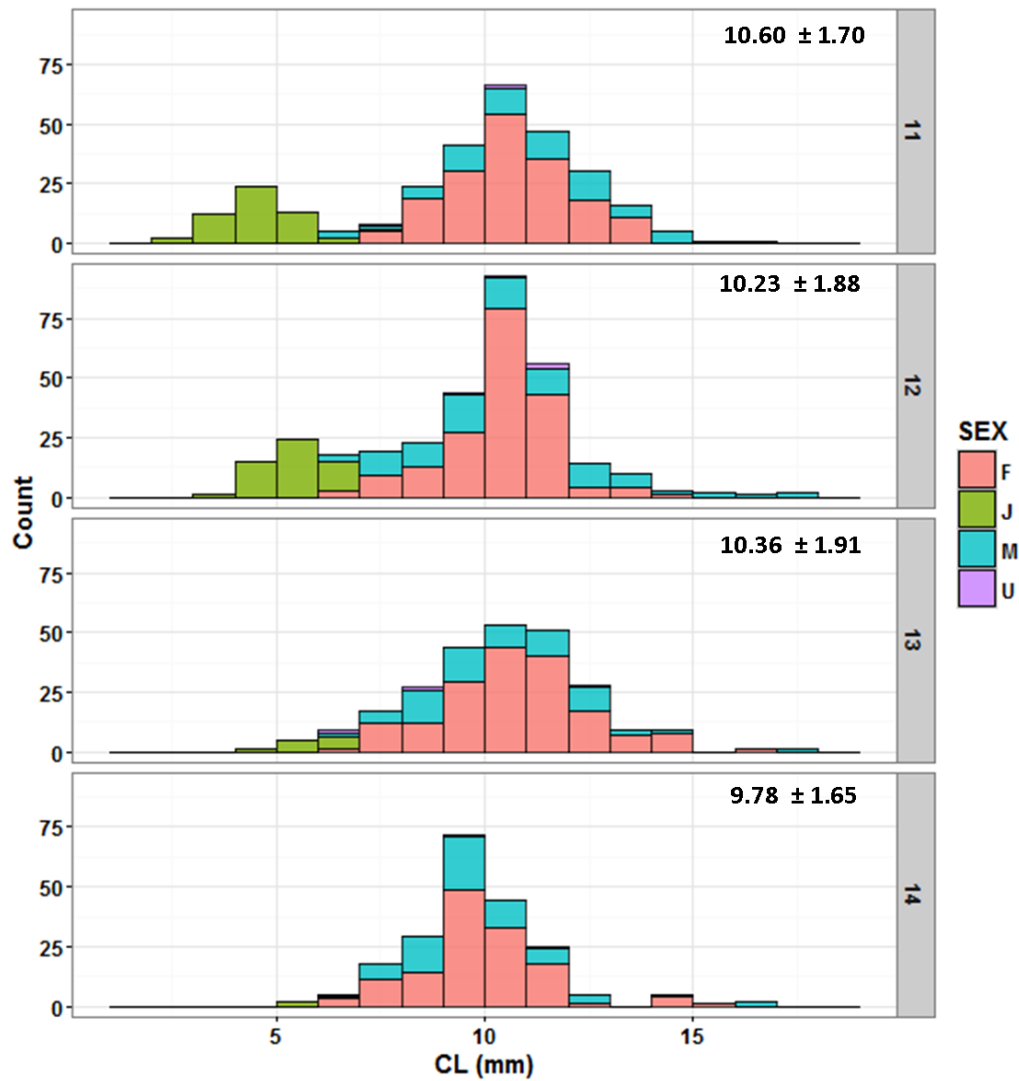
Figure 4.5 Results for model analysis of lipofuscin frequency data from annual surveys using the 'mixdist' package in R (McDonald 2012). Age class assignments and proportions are shown in table 4.5. Red lines denote best fitting mixture components with means shown as triangles on the x-axis. The green line denotes full model fit to LF Index frequency distribution. Age classes are shown with roman numerals and were selected based on LF accumulation rates recorded for *N. californiensis* (Colman et al, unpublished data).
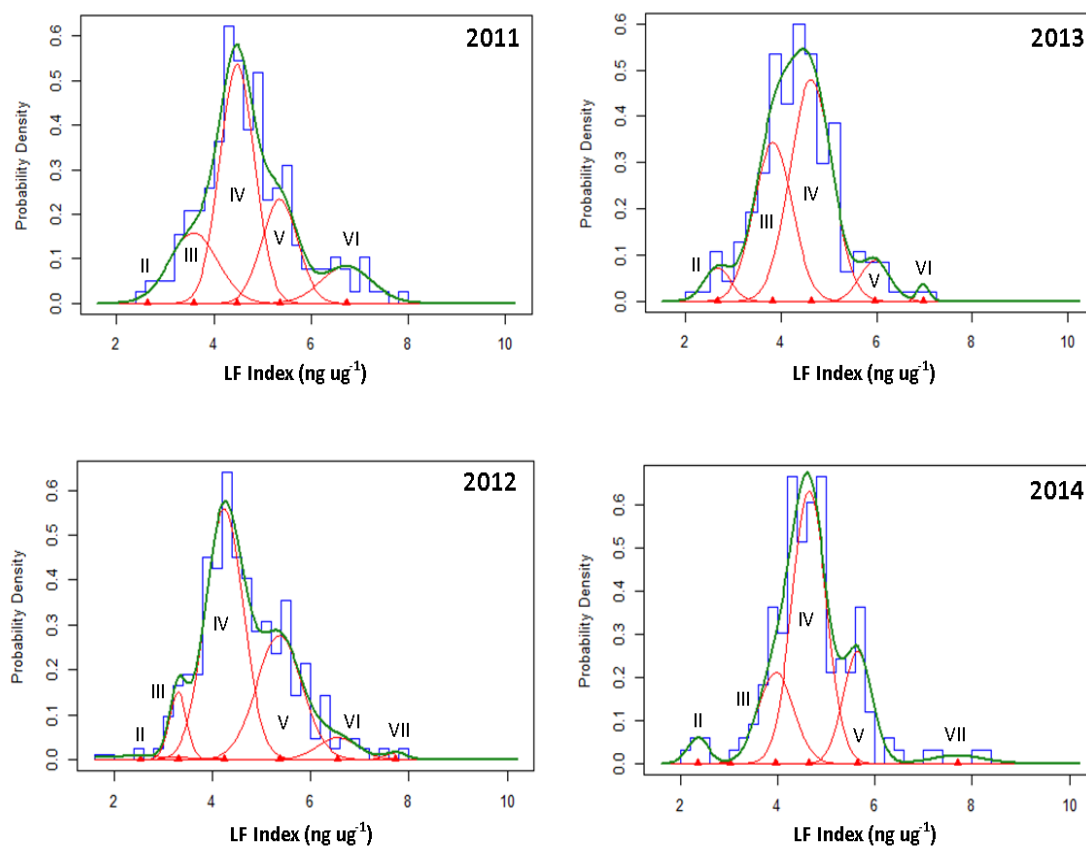
Figure 4.6 Plot showing numbers-at-age of *N. californiensis* for each cohort based on lipofuscin concentration from shrimp collected in population surveys from 2011-2014. Numbers-at-age were estimated by dividing the total abundance by portions in each age class determined with modal analysis (fig 4.5). Colors denote cohorts as they advance through age classes.
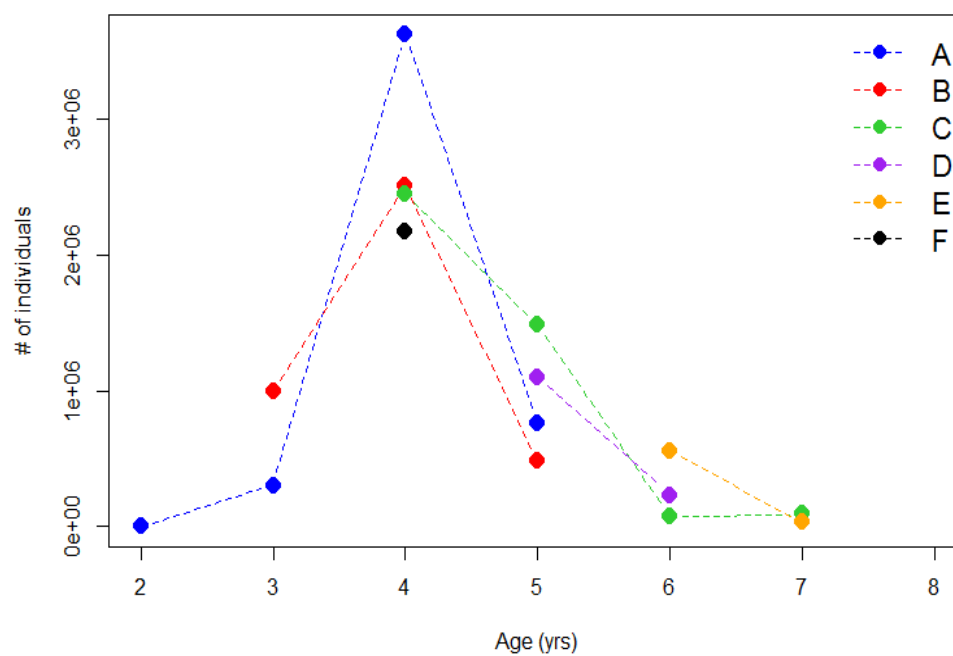
Figure 4.7 Plot showing ANCOVA results used to estimate mortality rate using equation 2. Mortality rate was not found to be statistically different among cohorts. Different color points represent numbers-at-age for separate cohorts as they progress through time. Error bars are standard error for estimates of numbers-age and shaded area represents 95% confidence interval for estimate of mortality rate from full model (Table 4.7).
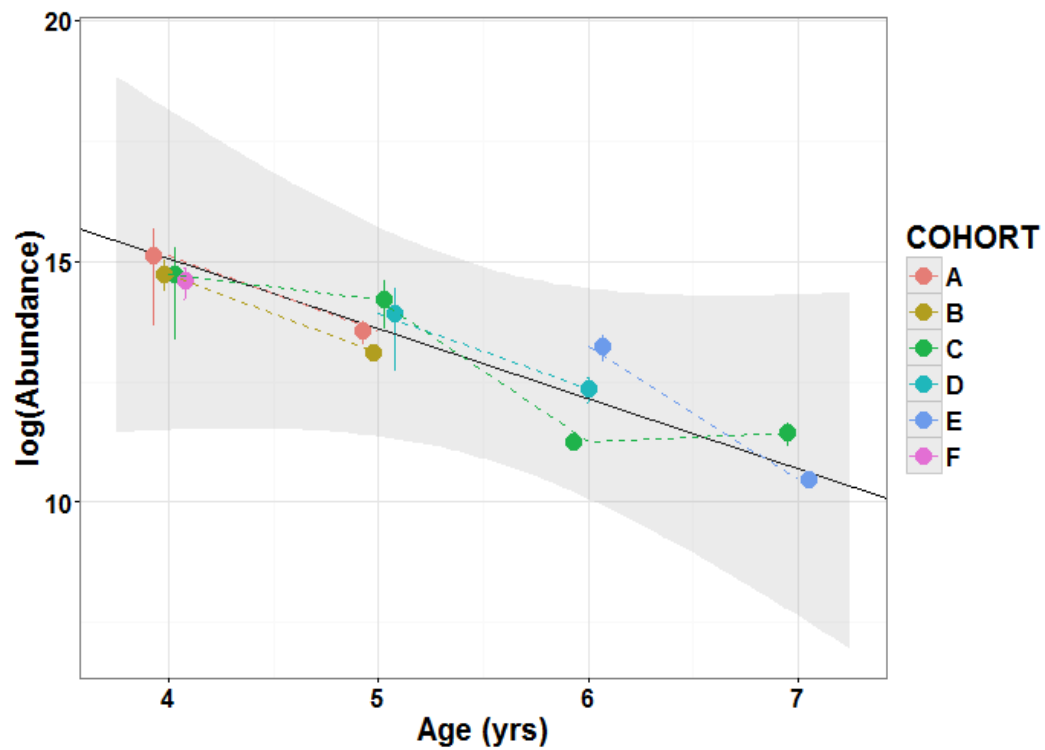
Figure 4.8 Prediction for changes in populaton density calcuated in model scenarios A) single cohort example under different mortality scenarios. Red line shows actual mortality with dashed line representing 95% confidence interval (B) Multi-cohort example with population projected toward equlibruim under different combinations of constant recrutiment and mortalty. In this scenario mortality was modeled at 0.2 yr $^{-1}$ and 0.8 yr $^{-1}$. Recruitment (at age 2 years) was modeled at 20 and 60 shrimp m^{-2}. Red line represents the actual average density for *N. californiensis* determined over the 4 year survey (94 shrimp m^{-2}).
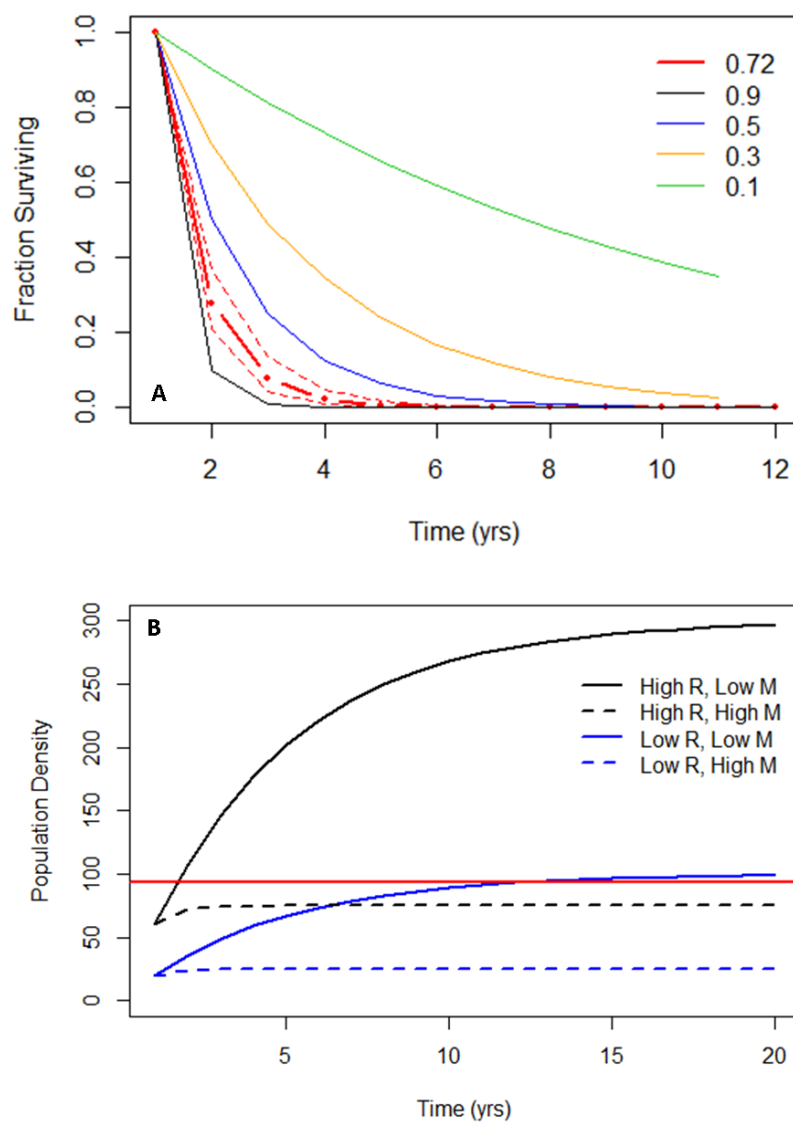
Figure 4.9 A) Negative bimomial probabity distribution for estimating numbers of 2 year old shrimp in monitoring surveys of *N. californiensis* in Yaquina Bay from 2005 – 2015 (Dumbauld unpublished). Bar plot shows real data and density curve represents the probably distribution function (mean = 11 and dispersion = 0.4). B) Population predictions from one interation of the stochastic recruitment scenario C) Results of 500 iterations of the stochastic recruitment model used to estimate average population density (14.98 shrimp m^{-2}).
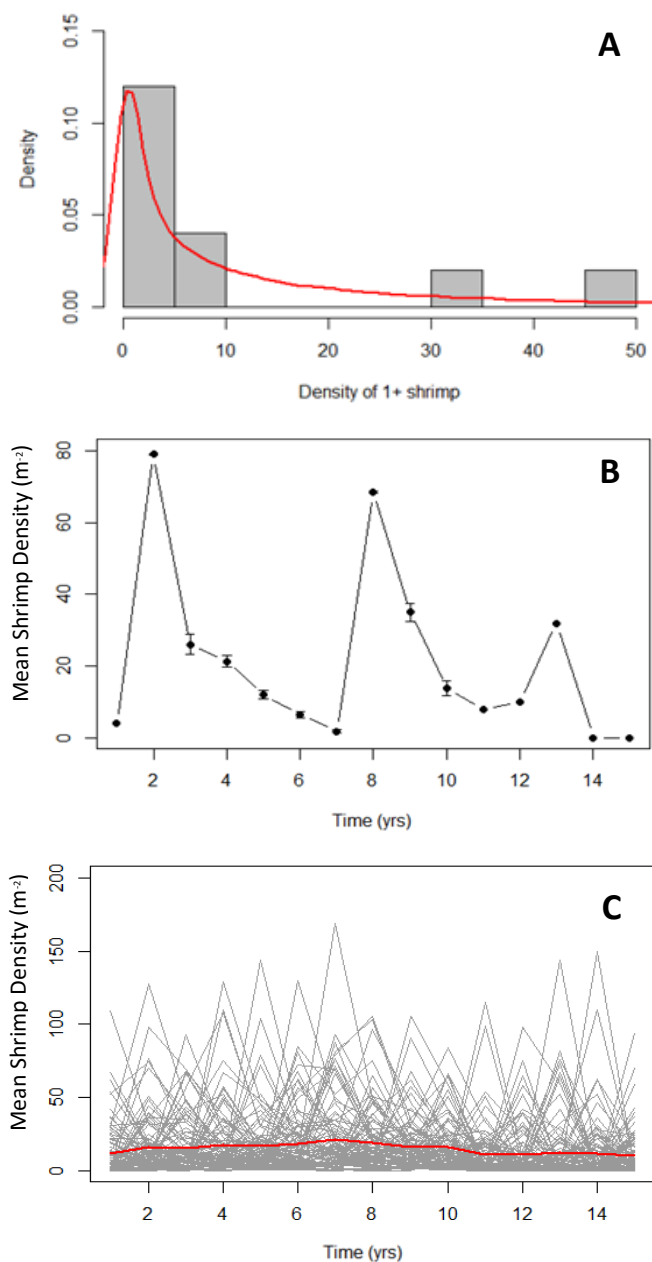
Table 4.1 Table showing population statistics from data collected during annual surveys of *N. californiensis* on Idaho Flats. Estimated population size from each survey year is based on IDW interpolations. S.D; Standard Deviation.

Year	N	Area (1000 m²)	Population Size (# 10⁶)	S.D.	% Pop Change	Ave Density (m⁻²)
2011	105	44.92	6.178	210,671	na	137.54
2012	154	56.26	5.293	237,458	-14.33	94.07
2013	145	91.60	7.098	242,289	+34.09	77.49
2014	100	58.07	3.955	830,276	-44.28	68.10

Table 4.2 Coefficients with standard errors from regression analysis the burrow number to shrimp number relationship from four years of surveys. * indicates statistically significant (p<0.05), d.f; degrees of freedom.

Coefficient	2011	2012	2013	2014	Pooled
Intercept	19.75 (16.55) *	28.38 (9.34) *	26.56 (12.80)*	12.26 (19.69)	21.63 (7.57) *
Burrows m^{-2}	0.51 (0.08)*	0.42 (0.04) *	0.34 (0.06) *	0.37 (0.10) *	0.41 (0.04)*
d.f.	8	9	9	8	40
adj r^2	0.8	0.914	0.733	0.572	0.752

Table 4.3 Table showing the proportion of shrimp classified as juveniles in each survey year

Size Class	2011	2012	2013	2014
< 6.0 mm	51	40	6	2
>=6.0	244	285	249	206
% Juveniles	0.173	0.123	0.024	0.010

Table 4.4 Sex ratios estimated from population survey data with results from chi-squared tests for equal proportions.

SEX	2011	2012	2013	2014
F	174	200	177	136
M	67	108	79	71
F:M RATIO	2.60	1.85	2.24	1.92
X^2	8.741	1.125	1.831	0.183
p	0.003	0.288	0.176	0.668

Table 4.5 Estimates for the proportion of *N. californiensis* in each age class determined with the 'mixdist' model on LF Index frequency distributions. Only shrimp > 6.5 mm were used in this analysis. pi; proportion of individuals in each distribution, mu; mean of normal components, sigma; standard deviation of normal components, Est. age; estimated age as from mean LF Index.

2011

pi (S.E.)	mu (S.E)	sigma (S.E)	Est. age (yrs)
<0.0001 (0.0001)	2.626 (56.127)	0.384 (34.063)	2
0.196 (0.177)	3.585 (0.457)	0.4942 (0.175)	3
0.480 (0.549)	4.481 (0.283)	0.3571 (0.277)	4
0.215 (0.472)	5.358 (0.674)	0.3678 (0.561)	5
0.109 (0.067)	6.728 (0.418)	0.5198 (0.200)	6
		X^2_{20}	14.723
		p	0.790

2012

pi (S.E.)	mu (S.E)	sigma (S.E)	Est. age (yrs)
0.015 (0.060)	2.539 (4.419)	0.857 (2.938)	2
0.066 (0.048)	3.301 (0.108)	0.175 (0.077)	3
0.540 (0.310)	4.243 (0.195)	0.386 (0.156)	4
0.320 (0.451)	5.364 (0.272)	0.463 (0.631)	5
0.050 (0.191)	6.546 (1.510)	0.409 (0.721)	6
0.008 (0.009)	7.733 (0.237)	0.203 (0.144)	7
		X^2_{15}	17.342
		p	0.298

2013

pi (S.E.)	mu (S.E)	sigma (S.E)	Est. age (yrs)
0.051 (-)	2.663 (-)	0.280 (-)	2
0.346 (0.552)	3.826 (0.836)	0.401 (-)	3
0.523 (0.762)	4.625 (0.665)	0.4353(0.3775)	4
0.070 (0.041)	5.958 (0.207)	0.3134(0.1137)	5
0.011 (0.007)	6.979 (-)	0.121 (-)	6
		X^2_{8}	12.37
		p	0.135

2014

pi (S.E.)	mu (S.E)	sigma (S.E)	Est. age (yrs)
0.032 (0.014)	2.362 (0.110)	0.216 (0.135)	2
0.194(0.183)	3.966 (0.259)	0.365 (0.112)	3
0.555 (0.232)	4.646 (0.122)	0.350 (0.149)	4
0.196 (0.081)	5.645 (0.139)	0.299 (0.111)	5
0.024 (0.0135)	7.699 (0.406)	0.516 (0.33)	7
		X^2_{21}	28.14
		p	0.136

Table 4.6 Life table showing numbers-at-age determined for each cohort sampled over the 4-year survey with standard errors. These data were used to in the ANCOVA to estimate annual mortality rate from equation 2. Numbers-at-age and error are reported in millions (10^6).

Year	4	5	6	7
2011	2.452 (1.823)	1.100 (0.766)	5.560 (0.152)	
2012	2.508 (0.790)	1.486 (0.681)	0.234 (0.068)	.036 (0.002)
2013	3.624 (2.770)	0.483 (0.027)	.0762 (0.003)	
2014	2.172 (0.706)	0.765 (0.185)		.092 (0.224)

Table 4.7 Coefficients from linear model of log-transformed numbers-at-age used to estimate mortality rate. A sums of squares F-test was used compare the full model containing cohort and time with a reduced model containing time only.

	Time Only	Time + Cohort
	Estimate (S.E.)	Estimate (S.E.)
Log (Abundance)		
Intercept	19.981 (0.781)*	20.854 (1.241)*
Time (yrs)	-1.281 (0.146)*	-1.451 (0.255)*
CohortB	-	-0.415 (0.674)
CohortC	-	0.025 (0.636)
CohortD	-	0.261 (0.720)
CohortE	-	0.429 (0.844)
CohortF	-	-0.460 (0.835)
N	13	13
adj R^2	0.863	0.797
$F_{5,11}$		0.285
p		0.905

CHAPTER 5

SUMMARY AND FUTURE RESEARCH DIRECTIONS

The findings presented in this dissertation provides insight into how two co-occurring species of thalassinidean shrimps persist in a dynamic estuarine environment and highlights how they are different with respect to their age and growth dynamics. Investigation of diets indicated that *N. californiensis* and *U. pugettensis* feed on the same general mix of food sources, but the greater spatial extent in which *N. californiensis* populations are found results in a more diverse diet. Some food sources, such as marine phytoplankton and red algae, were found to be potentially important in controlling condition and likely growth of *N. californiensis*, which may have population level consequences as diet is an important factor in reproduction and offspring survival. This work also provided evidence that environmental factors likely play a role in the growth of burrowing shrimp in Yaquina Bay. Like many other poikilothermic species, temperature appeared to play a role determining growth rate for *U. pugettensis* and when temperature data are available, it is possible to predict patterns of growth for this species. Food effects were important for growth in *N. californiensis* but temperature did not strongly influence growth or physiological aging rate.

Analysis of lipofuscin has shown promise for estimating age in the burrowing shrimp, *N. californiensis,* and the work presented here confirms that the method can overcome challenges in estimating age using traditional length-based methods. Growth rates in *N. californiensis* were significantly different across sites while lipofuscin accumulation rate was similar. This finding provided support for and enabled the lipofuscin aging method to be used across a broad spatial area to empirically derive mortality rates for the shrimp population in Yaquina Bay. Our empirical estimate for mortality can now be applied to explore the population dynamics of *N. californiensis* using a discrete-time, age-structured model and inform management of the species across a broad spatial scale.

This dissertation research represents the "next generation" of population studies on thalassinidean shrimp, integrating a number of methodologies to understand the age and growth dynamics for members of this unique taxonomic group. In addition to achieving a greater understanding of burrowing shrimp population dynamics, this study revealed several potential avenues for future research. One hypothesis to come out of the diet study (Chapter 2) is that food quality plays an important role in growth and possibly reproduction of burrowing shrimp. Identification of shrimp diets has provided the information necessary to implement manipulative feeding studies with the aim of developing a mechanistic understanding of food quality effects on individual growth and offspring quality.

This study also provided additional support for the hypothesis that *N. californiensis* has developed metabolic pathways to cope with a highly dynamic thermal and nutritional environment (Chapter 3). Future studies could be conducted to explicitly test metabolic response to environmental stressors (i.e., temperature, osmotic stress, starvation). Understanding the metabolic demands of the shrimp and also their response to changes in the environment will prove to be useful in describing habitat preferences and potential shifts in the species range with future changes to the regional climate.

Data from the broad scale population surveys and lipofuscin-based age structure revealed interesting patterns in the spatial structure of the *N. californiensis* population in Yaquina Bay. While recruitment and mortality are clearly important in predicting population abundance, the mosaic of shrimp densities across the tidal flat seen in the broad survey supports the hypothesis that migration of juveniles may be an important mechanism in regulating shrimp populations. Very little work has been done to capture movement of adults and juveniles or to understand the cues that would cause shrimp to move. This is an area of research that will be important for future management of shrimp as pests to oyster production under integrated pest management (IPM). Understanding the spatial extent in which juveniles are found and the conditions under which they move will add additional information that can be incorporated into an IPM plan to predict future threats from shrimp infestation.

Lipofuscin has been used to estimate chronological age and population age-structure in a number of crustacean species, however, the work presented in Chapter 3 showed that the method may not be able to be universally applied. Lipofuscin index did not correlate to age in the mud shrimp, *U. pugettensis*, and this could be attributed to the indeterminant growth pattern exhibited in mud shrimp where they continue to grow relatively quickly throughout their lifespan. In contrast, *N. californiensis* appears to grow slowly or as environmental conditions allow until they reach a point where growth is slowed or stopped altogether. It is under these conditions that lipofuscin aging has a great advantage over traditional size-based methods. Following the methods described in this dissertation, aging studies can be implemented with other crustacean species to investigate the life history strategies and growth pathways where lipofuscin provides more robust age estimates than size-based methods. This information could be used to develop a general framework to describe when alternative aging methods, specifically lipofuscin, should be considered for the implementation of crustacean population studies.

Overall, the lipofuscin method has the ability to overcome challenges of variable growth in estimating age in crustaceans. The method continues to show promise for application to species where economic and ecological interests require greater understanding of population structure and population dynamics for the development of management plans. The findings and methodologies presented in this dissertation can be used to inform others seeking to address similar challenges in understanding population ecology of crustaceans around the globe and contributes to the next generation of invertebrate population studies.

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APPENDICES

<u>APPENDIX A</u>

Supplemental material for Chapter 2:
Output summaries of statistical models

Table A1 Correlations of FA biomarkers to non-metric multidimensional scaling (NMDS) ordination axes of fatty acid compositions for *N. californiensis* and *U. pugettensis*. Biomarker compositions defined in Table 2.8.

N. californiensis

Biomarker	Axis 1	Axis 2	R^2	*p*
Total FA	0.39	0.92	0.78	0.00
Diatom1	-0.79	0.61	0.90	0.00
Diatom2	-1.00	0.07	0.96	0.00
Bacterial	-0.58	-0.82	0.80	0.00
Dinoflagellate 1	0.58	-0.82	0.96	0.00
Dinoflagellate 2	0.98	0.17	0.97	0.00
Eelgrass	0.79	-0.61	0.97	0.00
Macrophyte	-0.65	-0.76	0.86	0.00
Chlorophyte	-0.72	-0.69	0.83	0.00
Rhodophyte	0.42	0.91	0.81	0.00

U. pugettensis

Biomarker	Axis 1	Axis 2	R^2	*p*
Total FA	-0.79	0.61	0.43	0.00
Diatom1	-0.98	-0.21	0.97	0.00
Diatom2	-0.73	-0.69	0.97	0.00
Bacterial	0.95	0.31	0.69	0.00
Dinoflagellate 1	0.99	0.12	0.93	0.00
Dinoflagellate 2	0.60	0.80	0.89	0.00
Eelgrass	0.78	0.63	0.83	0.00
Macrophyte	0.77	-0.64	0.70	0.00
Chlorophyte	0.06	-1.00	0.61	0.00
Rhodophyte	-0.96	0.29	0.74	0.00

Table A2 Posterior probability distributions of proportional diet compositions from five source Stable Isotope Analysis in R (SIAR) model for *N. californiensis* and *U. pugettensis*. Sites are ordered top to bottom corresponding to increasing distance from estuary mouth. SWI; Seawall Island, IF; Idaho Flats, RAC; Raccoon Flats, UPR; Upriver, TI; Tongue Island.

	N. californiensis					*U. pugettensis*			
	SWI					IF			
	Mean	SD	max	min		Mean	Mode	max	min
eelgrass	11.7	2.6	19.2	0.2		8.4	5.2	31.8	0.0
epiphyte	12.6	7	45.9	0		24.8	8.8	58.4	0.0
pom	44.1	8.5	78.6	10.9		30.0	7.8	66.2	0.1
spom	19.9	8.3	56.1	0		23.7	4.5	43.5	3.6
ulva	11.7	7	47.4	0		13.1	7.0	44.7	0.0
	IF					RAC			
	Mean	SD	max	min		Mean	SD	max	min
eelgrass	6.1	5.6	43.6	0.0		8.5	5.3	34.7	0.0
epiphyte	29.8	15.2	84.1	0.0		24.5	8.9	59.0	0.1
pom	30.2	6.6	48.0	1.2		30.2	7.8	61.8	0.1
spom	8.9	8.1	55.0	0.0		23.6	4.5	41.8	5.1
ulva	24.9	10.5	53.4	0.0		13.2	7.0	40.7	0.0
	TI					UPR			
	Mean	SD	max	min		Mean	SD	max	min
eelgrass	8.3	4.2	21.7	0.0		2.4	2.0	19.4	0.0
epiphyte	20.0	9.1	66.0	0.0		20.1	9.3	55.6	0.0
pom	40.6	5.8	67.0	13.7		46.3	12.5	96.4	0.7
spom	17.8	4.3	34.9	0.1		24.7	5.7	46.7	1.8
ulva	13.3	7.2	39.5	0.0		6.6	5.1	44.8	0.0

APPENDIX B

Supplemental material for Chapter 3: Data tables

Figure B1 Temperature measurements from Sally's Bend field site taken at 15 minute intervals. Dotted lines represent the three temperatures tested in temperature trials (10° C, 13°C, and 16°C). Data continuously recorded with HOBO ® temperature probes deployed at 10 cm below sediment surface.
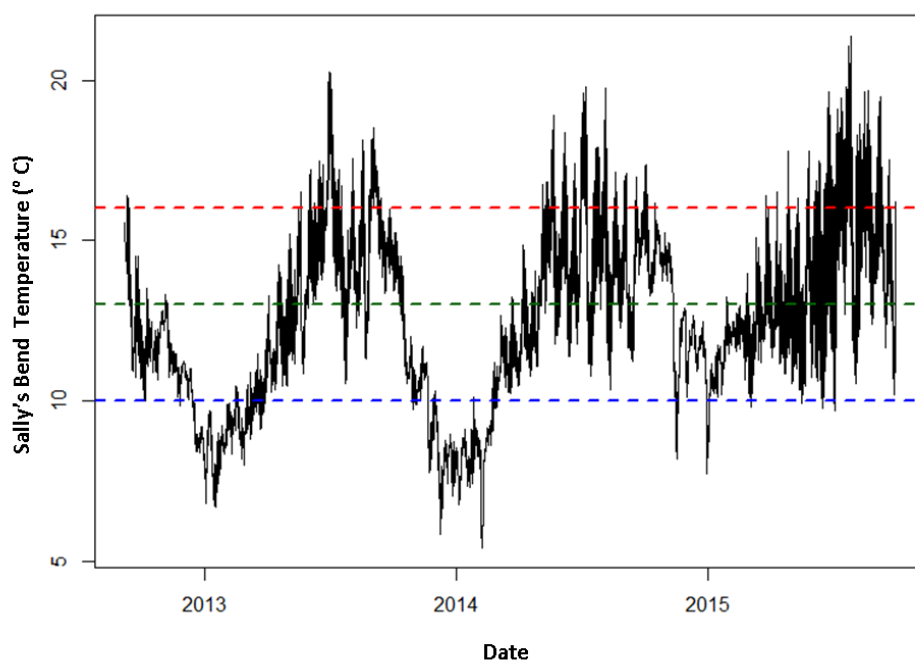
Figure B2 Scatterplot showing relationship of carapace length to log (LF Index) for *U. pugettensis* from field growth experiment. Colors denote cohorts as determined by modal analysis of length frequency data. Trend line represents significant linear regression (p = 0.0035, adj r^2 = 0.21).
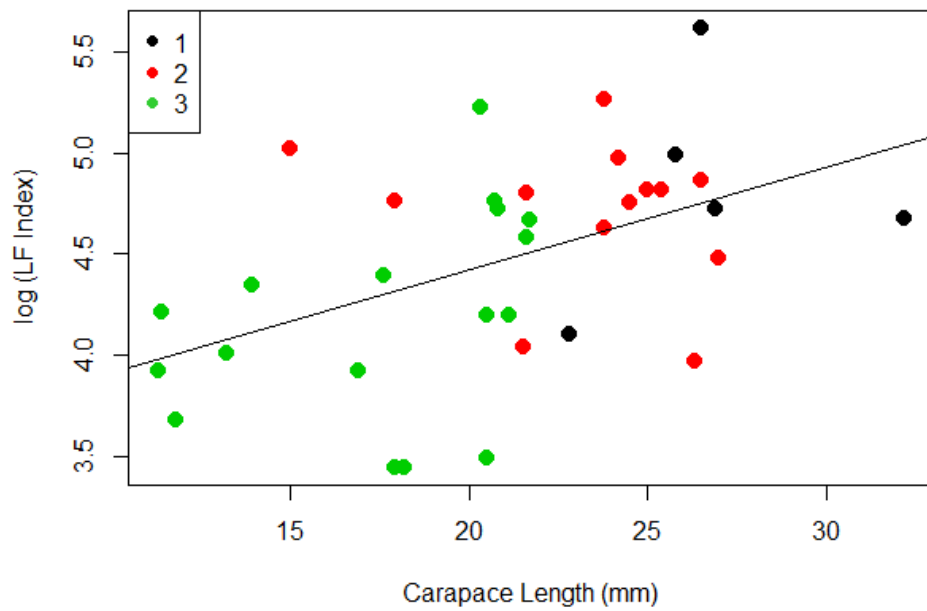
Table B1 Table showing mean carapace length (CL) and survival for *N. californiensis* from temperature trials. CL in; initial CL, CL out; final CL, SD; standard deviation

Trial	Tank	Treatment	N	CL In (SD)	CL Out (SD)	Δ CL	% survival
	INITIAL	INITIAL	25	8.44 (0.56)	-	-	-
	D	10	20	8.60 (0.55)	8.72 (0.63)	0.12	83
	E	10	19	8.30 (0.67)	8.43 (0.68)	0.13	83
1	A	13	15	8.46 (0.60)	8.81 (0.76)	0.36	75
	F	13	19	8.37 (0.62)	8.61 (0.67)	0.24	86
	B	16	20	8.46 (0.53)	8.92 (0.48)	0.46	95
	C	16	20	8.46 (0.66)	8.38 (0.69)	-0.08	95
	INITIAL	INITIAL	20	10.88 (1.05)	-	-	
	D	10	19	10.38 (0.97)	10.46 (1.07)	0.08	95
	E	10	18	10.70 (1.06)	10.92 (0.88)	0.22	90
2	B	13	10	10.67 (1.15)	10.37 (1.09)	-0.30	50
	F	13	18	10.84 (0.91)	10.77 (0.70)	-0.07	90
	A	16	19	10.64 (0.95)	10.65 (0.98)	0.01	95
	C	16	15	10.91 (0.80)	11.03 (0.94)	0.12	75

Table B2 Table of mean LF Index and change in LF Index for *N. californiensis* from temperature trials. LF Index values are tank means. SD; standard deviation

Trial	Tank	Treatment	N	LF Index (SD)	Δ LF
1	INITIAL	INITIAL	23	2.18 (0.77)	-
	D	10	19	3.57 (1.11)	1.39
	E	10	18	3.15 (0.64)	0.97
	A	13	14	2.9 (0.67)	0.72
	F	13	18	3.68 (0.78)	1.5
	B	16	20	2.86 (0.65)	0.68
	C	16	20	2.5 (0.66)	0.32
2	D	10	19	3.01 (0.63)	0.83
	E	10	18	2.77 (0.53)	0.59
	B	13	19	2.6 (0.58)	0.42
	F	13	18	2.79 (0.7)	0.61
	A	16	10	1.98 (0.38)	-0.2
	C	16	15	2.26 (0.76)	0.08

Table B3 Table showing mean carapace length (CL) and survival for *U. pugettensis* from temperature trials. CL in; initial CL, CL out; final CL, SD; standard deviation

Tank	Treatment	N	CL In (SD)	CL out (SD)	Δ CL	% survival
INITIAL	INITIAL	25	13.58 (1.37)	-	-	-
D	10	9	13.85 (1.11)	14.14 (1.05)	0.60	45
E	10	6	13.34 (0.97)	12.88 (1.64)	-0.27	30
A	13	6	13.98 (1.01)	15.10 (1.85)	1.22	30
F	13	6	13.81 (1.19)	14.08 (1.19)	0.00	30
B	16	2	13.91 (0.94)	14.80 (0.99)	0.80	10
C	16	5	13.66 (1.02)	13.72 (0.98)	0.42	25

Table B4 Table showing mean LF Index and change in LF Index for *U. pugettensis* from temperature trials. LF Index values are tank means. SD; standard deviation

Tank	Treatment	N	LF Index (SD)	Δ LF
INITIAL	INITIAL	25	3.99 (1.43)	-
D	10	7	5.58 (1.68)	1.59
F	10	6	5.43 (1.14)	1.45
A	13	6	6.11 (0.86)	2.12
C	13	5	6.75 (0.48)	2.76
B	16	0	-	-
E	16	6	6.66 (1.43)	2.68

Table B5 Table of mean growth rates and mean LF index values for *N. californiensis* from field study locations. TDD; temperature degree-days, Days; calendar days elapsed, Δ CL; change in carapace length, SD; standard deviation, TI; Tongue Island, IF; Idaho Flats, SB; Sally's Bend.

Date	Site	Days	TDD	N	Δ CL (SD)	LF Index (SD)
1/27/2014	TI	525	-	8	3.48 (1.16)	-
2/3/2014	IF	573	1082.44	18	3.64 (0.80)	-
2/4/2014	SB	515	936.32	18	4.36 (1.20)	-
8/11/2014	IF	762	1421.99	24	4.00 (0.90)	2.13 (0.36)
8/14/2014	SB	706	1218.91	23	4.54 (0.94)	2.19 (0.74)
2/24/2015	SB	900	1757.48	31	5.51 (1.12)	2.59 (0.54)
2/25/2015	IF	960	1988.63	18	4.18 (1.56)	3.38 (3.38)
10/1/2015	SB	1119	2570.79	32	6.45 (1.11)	3.26 (1.43)
10/9/2015	IF	1186	2988.35	21	4.74 (1.20)	4.36 (1.38)

Table B6 Table of mean growth rates and mean LF index values for *U. pugettensis* from field enclosures in Idaho Flats. TDD; temperature degree-days, Days; calendar days elapsed, CL; mean carapace length, SD; standard deviation; LF; mean LF Index

Date	Days	TDD	Cohort	Bucket	N	CL	SD CL	LF	SD LF
5/18/2009	0	0	1	INITIAL	30	12.80	1.15	-	-
10/16/2009	151	363.2	1	10	7	15.53	3.08	-	-
10/16/2009	151	363.2	1	5	14	16.54	2.06	-	-
10/16/2009	151	363.2	out	10	1	26.60	-	-	-
10/16/2009	151	363.2	out	5	1	27.50	-	-	-
2/24/2010	282	470.9	1	7	9	17.58	3.74	-	-
2/24/2010	282	470.9	1	8	5	17.46	2.13	-	-
2/24/2010	282	470.9	2	7	1	9.60	-	-	-
2/24/2010	282	470.9	out	7	1	30.00	-	-	-
6/3/2010	381	619.6	1	11	8	19.84	1.69	-	-
6/3/2010	381	619.6	1	6	8	20.10	2.62	-	-
6/3/2010	381	619.6	2	11	5	12.54	1.09	-	-
6/3/2010	381	619.6	out	6	4	28.55	0.62	-	-
12/2/2010	563	919.7	1	2	10	23.84	2.90	-	-
12/2/2010	563	919.7	1	X	12	22.82	1.55	-	-
12/2/2010	563	919.7	2	2	4	15.63	3.87	-	-
12/2/2010	563	919.7	2	X	7	16.41	2.19	-	-
6/8/2011	751	1017.6	1	12	5	26.84	3.40	6.08	1.18
6/8/2011	751	1017.6	1	X	7	25.69	1.71	-	-
6/8/2011	751	1017.6	2	12	6	18.35	2.71	7.09	1.32
6/8/2011	751	1017.6	2	X	2	19.15	3.61	-	-
6/8/2011	751	1017.6	3	12	5	12.32	1.16	4.82	0.75
6/8/2011	751	1017.6	3	X	4	12.28	1.23	-	-
12/6/2011	932	1393.96	2	A	4	24.60	0.73	3.92	0.25
12/6/2011	932	1393.96	2	B	6	25.85	1.36	4.41	1.38
12/6/2011	932	1393.96	3	A	11	20.02	1.60	3.92	1.24
12/6/2011	932	1393.96	3	B	2	17.75	0.21	4.92	-
12/6/2011	932	1393.96	4	A	18	8.42	1.78	-	-
12/6/2011	932	1393.96	4	B	11	8.35	0.95	-	-

APPENDIX C

Supplemental material for Chapter 4: Population model output

Table C1 Model output from combined equilibrium scenarios (Figure 4.8B). R = 20 (L) and 60 (H) shrimp m^{-2}. M = 0.2 (L) and 0.8 (H) yr^{-1}.

Time	High R, Low M	High R, High M	Low R, Low M	Low R, High M
1	60.0	60.0	20.0	20.0
2	108.0	72.0	36.0	24.0
3	146.4	74.4	48.8	24.8
4	177.1	74.9	59.0	25.0
5	201.7	75.0	67.2	25.0
6	221.4	75.0	73.8	25.0
7	237.1	75.0	79.0	25.0
8	249.7	75.0	83.2	25.0
9	259.7	75.0	86.6	25.0
10	267.8	75.0	89.3	25.0
11	274.2	75.0	91.4	25.0
12	279.4	75.0	93.1	25.0
13	283.5	75.0	94.5	25.0
14	286.8	75.0	95.6	25.0
15	289.4	75.0	96.5	25.0
16	291.6	75.0	97.2	25.0
17	293.2	75.0	97.7	25.0
18	294.6	75.0	98.2	25.0
19	295.7	75.0	98.6	25.0
20	296.5	75.0	98.8	25.0