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NORTH PACIFIC RESEARCH BOARD FINAL REPORT

The effects of ocean acidification on maternal condition and reproductive success and larval condition and survival of Tanner crabs, *Chionoecetes bairdi*.

NPRB Project #1010 Final Report

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August 2014

Abstract

The increase in atmospheric CO_2 concentrations, caused by the burning of fossil fuels and concrete production, has caused a corresponding increase in the CO_2 concentrations in the ocean. This has changed the carbonate chemistry of the oceans and decreased the pH. Continued increases in CO_2 levels and subsequent ocean pH drop will likely affect physiological processes such as growth, survival, reproduction, and behavior in marine organism. Calcifying organisms may be particularly affected as the reduction in pH makes it more difficult to excrete and sustain a calcified shell or exoskeleton. We examined the effects of ocean acidification on the embryo and larval stages of the economically important southern Tanner crab, *Chionoecetes bairdi*. Ovigerous females were reared in one of 3 treatments: ambient pH (~8.1), pH 7.8, and pH 7.5 for 2 years. Embryos and larvae in year 1 were from oocytes developed in the field whereas embryos and larvae in year 2 were from oocytes developed under acidified conditions. Larvae hatched each year, were also exposed to 3 pH treatments to examine starvation-survival, morphology, condition, and calcium/magnesium content. Embryo development in ambient and pH 7.8 treatments differed from the pH 7.5 treatment. Exposure to acidified conditions at the larval stage alone had minimal effects on the larvae, however, larvae exposed during oogenesis and embryogenesis differed morphometrically, were smaller, and had lower calcium and magnesium contents. This research highlights the importance of focusing exposure experiments on vulnerable life history stages and indicates that ocean acidification will likely affect commercial crab stocks.

Key Words

Southern Tanner crab, *Chionoecetes bairdi*, Bering Sea, embryological development, survival, calcification, experimental CO_2 dosing, ocean acidification, reproduction.

Citation

Foy, R.J., W.C. Long, K. Swiney. 2014. The effects of ocean acidification on maternal condition and reproductive success and larval condition and survival of Tanner crabs, *Chionoecetes bairdi*. NPRB Project 1010 Final Report, 68 pages.

This work has been subsequently published in the peer-review literature:

Swiney, K. M., Long, W. C., and Foy, R. J. 2016. Effects of high pCO2 on Tanner crab reproduction and early life history, Part I: long-term exposure reduces hatching success and female calcification, and alters embryonic development ICES Journal of Marine Science, 73: 825-835.

Long, W. C., Swiney, K. M., and Foy, R. J. 2016. Effects of high pCO2 on Tanner crab reproduction and early life history, Part II: carryover effects on larvae from oogenesis and embryogenesis are stronger than direct effects. ICES Journal of Marine Science, 73: 836-848.

Table of Contents

Study Chronology

This study was approved for funding in May 2010 and funds were made available in October 2010. Project reports were submitted in January and July 2011, 2012, and 2013. In January 2013 a no-cost extension was granted to the project with some minor changes to the initial objectives. To test the effects of ocean acidification on the ovary development, subsequent embryo development, and subsequent larval development stages of Tanner crab, the project end date was extended until January 31, 2014. Adults exposed during the first year of the project were exposed for a second reproductive cycle thus increasing the scope of the project to include ovary development and provide a whole early life history perspective on the effects of ocean acidification. This project was completed simultaneous to a NOAA Ocean Acidification Program funded series of studies to assess the effects of ocean acidification on king and Tanner crabs in Alaska.

Introduction

Dissolution of anthropogenic CO_2 has reduced global mean surface water 0.1 pH units below preindustrial levels, a change of about 26% (Caldeira & Wickett 2003, Orr et al. 2005). In addition, deep oceanic waters are depleted in carbonate due to respiration resulting in a saturation depth below which calcium carbonate dissolves. Thus, decreased carbonate ion concentration hinders the formation of shells and support structures by some calcifying organisms (Caldeira & Wickett 2003, Feely et al. 2004, Orr et al. 2005). Crustaceans are calcifying organisms that are critical to marine food webs and support important commercial fisheries. In the North Pacific Ocean, where the saturation depth is relatively shallow due to the cold temperature and age of advected deep water masses, southern Tanner crab *Chionoecetes bairdi* (hereafter referred to as Tanner crab) are ecologically and economically important crustaceans. The influence of lower pH and decreased carbonate ion concentration in seawater on the condition, survival, and shell calcium carbonate content of Tanner crabs in Alaska is unknown.

Adult Tanner crab inhabit depths from the subtidal to 437 m and are thus affected by both surface increases in CO_2 and by deep undersaturated waters. Pubescent Tanner crab females about to undergo their molt to maturity are found in shallow water (<13m) (Stevens et al. 1993) and are thus exposed to surface water increases in CO_2 and subsequent decrease in pH. Multiparous females (brooding second or subsequent egg clutch) that must first hatch their larvae before extruding a new clutch are found in aggregations in deep water (approximately 150 m) which suggests that there is a migration of crabs toward deeper waters with age (Stevens et al. 1993). This migration may be impeded by shoaling of the saturation depth.

Acidified waters can have a significant effect on the development (Findlay et al. 2009, Parker et al. 2009), development time (Findlay et al. 2009), viability (Kurihara et al. 2004a), and even behavior (Ellis et al. 2009) of the embryos of marine invertebrates (though see Arnold et al. 2009). Further, acidified waters can reduce fertilization success (Parker et al. 2009), the hatching success of embryos (Kurihara et al. 2004a), and the fecundity of females (Kurihara et al. 2004b). Fecundity estimates for Tanner crabs range from 24,000 to 318,000 eggs (Hilsinger 1976) with primiparous crabs (brooding their first egg clutch) producing 62% (Wenner et al. 1991) to 70% (Somerton and Meyers 1983) as many eggs as equal-sized multiparous females.

Embryo development of northern Gulf of Alaska Tanner crab has been examined by both developmental staging and mean egg area measurements (Swiney 2008). Embryos of primiparous and multiparous females take approximately 2 months after egg extrusion to develop to the gastrula stage at which point they go into a state of embryonic developmental diapause, a period of arrested development. Embryonic developmental diapause lasts for approximately 6 months for primiparous females and 3 months for multiparous females and after diapause, embryos develop rapidly until larval hatching. Similar trends are observed with mean egg areas (Swiney 2008). Average larval hatching duration among Tanner crabs varies between 7 days for primiparous females and 12 days for multiparous females (Swiney 2008). The duration of marine invertebrate larval hatching is innate, heritable and not easily altered by most environmental influences (Morgan 1995); however larval hatching has not been examined with crabs reared in acidified water.

We hypothesized that ocean acidification would lead to a significant effects at the embryo and larval stages of Tanner crab. Specifically exposure to decreased pH (increased pCO_2) at the embryo stage will decrease fecundity, embryo viability, condition, hatching success, and maternal condition index while increasing embryo development time. At the larval stage, larval survival, mass, and condition will be decreased and larval morphology will be altered. We hypothesized that there will be reduced calcification in adult females and larvae.

Objectives

The overall goal of this study was to examine the effects of ocean acidification on aspects of the reproduction and larval development of Tanner crabs. Our objectives were to assess the effects of ocean acidification on 1) fecundity, embryo viability, embryo development, and hatching success; 2) larval

condition and survival; and 3) calcification in both the mothers and the larvae. The original study design was to expose adult female Tanner crab that had already extruded embryos (i.e. oocytes were developed *in situ*). In an approved amendment to the project, the experiment was extended through a second year to assess the effects of ocean acidification on fertilized embryos and larvae extruded from the same adult females that had been exposed to lower pH for approximately 12 months (i.e. oocytes were developed in ocean acidification conditions).

Effects of ocean acidification on fecundity, embryo viability, embryo development, and hatching success

We examined the effects of ocean acidification on fecundity, embryonic development, development time, embryo viability and hatching success in Tanner crabs in year 1 from ovigerous females caught near Kodiak Island and again in year 2 from the same adult females allowed to extrude clutches in the laboratory. The details of this objective are covered in the first manuscript developed as a result of this project (See Manuscript #1 on page 9). An experimental dosing system was developed at the NOAA Kodiak Laboratory to manipulate seawater carbonate chemistry. Females were placed into this system and randomly assigned to one of three acidification treatments based on projected future changes to ocean pH: 1) ambient pH (~8.1), 2) pH 7.8 (c. ~2100), and 3) pH 7.5 (c. ~2200) (Caldeira and Wickett, 2003). The experiment ran for two years and at the end of the experiment there were 10 females in the ambient treatment, 6 females in pH 7.8 treatments, and 7 females in the pH 7.5 treatments. A number of response variables were measured throughout the embryological and larval stages.

Effects of ocean acidification on larval condition and mortality

Similar to its effects on embryos, ocean acidification can also affect the survival (Talmage and Gobler 2009) and morphology (Kurihara et al. 2007, Dupont et al. 2008) of marine invertebrate larvae. More subtly, ocean acidification is likely to impose an energetic cost on developing larvae that would lead to a decrease in mass and changes in their chemical composition. The details of this objective are covered in the second manuscript developed as a result of this project (See Manuscript #2 on page 34). In this study, three sets of experiments were completed over three years. In the first year we used larvae that had been brooded in the wild to determine the effects on the larval stage only. We then held females with newly extruded embryos in the laboratory at three pH treatments, ambient and two experimental (pH 7.8 and pH 7.5), until the larvae hatched. In the second year we used these larvae, the embryos of which had developed under treatment conditions, to determine carryover effects of exposure at the embryo stage on larvae. In the third year, we held the females for another year in their pH treatments so that larvae that hatched out came from oocytes that developed in the females that were in treatment water and embryos

that had developed in treatment water. These larvae allowed us to determine carryover effects of exposure during both oogenesis and embryogenesis on larvae.

Effects of ocean acidification on calcification

Some marine invertebrates experience a decrease in calcification in response to ocean acidification (e.g. Maier et al. 2009), however, limited studies on decapods have found either a positive or no effect on calcification (Landes and Zimmer, 2012; Ries et al., 2009; Long et al., 2013). The details of this objective are covered in both of the manuscript produced from this project. To accomplish this objective, at the end of these experiments all adult females were sacrificed and a portion of their exoskeleton was assessed for calcium content. In the larval experiments the calcium and magnesium content was determined from a subset of larvae at the beginning of the experiment and a set of starvation experiments was also completed to collect calcium and magnesium content data at the end of the experiment.

Manuscripts

Manuscript #1: Ocean acidification alters embryo development and reduces the number of viable larvae hatched, and calcification in Tanner crab, *Chionoecetes bairdi*.

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Abstract

Ocean acidification, a decrease in ocean pH due to absorption of anthropogenic CO_2, has variable effects on different species. To examine the effects on Tanner crab (*Chionoecetes bairdi)* embryo development, number of viable larvae hatched, and calcification, ovigerous females were reared in one of 3 treatments: ambient pH (~8.1), pH 7.8, and pH 7.5 for 2 years. In general, embryo development in ambient and pH 7.8 treatments differed from the pH 7.5 treatment, but not each other. Differences in embryo morphology were slight in year one, averaging 3.6% (increases or decreases) between the pH 7.8 and 7.5 treatments. In year 2, the effects were larger and embryos in the pH 7.5 treatment had 10.1% larger yolks and 6.3% smaller embryos than the ambient treatment. The number of viable larvae hatched did not differ with treatment in year 1 and in year 2 did not differ between ambient and pH 7.8 treatments, but on average 71% fewer viable larvae hatched in the pH 7.5 treatment. After 2 years, percent calcium in female's carapaces was lower in pH 7.5 than the other treatments. Embryos and larvae in year 1 were from oocytes developed in the field whereas embryos and larvae in year 2 were from oocytes developed under acidified conditions (during year 1). Oocyte development appears to be sensitive to ocean acidification and effects carry over through embryo development and reduce the number of viable larvae hatched. The distressing effects from females exposed to acidified waters for the entire reproductive cycle (oocyte development to hatching) is apparent by the relatively small number of viable larvae hatched in the pH 7.5 treatment. Projected ocean pH levels within the next 2 centuries will likely have a pronounced impact on Tanner crab populations unless the crab are able to adapt to rapidly changing conditions. This project illustrates the need for long term ocean acidification studies.

Keywords: ocean acidification, Tanner crab, *Chionoecetes bairdi*, embryo development, hatching success, calcification

Introduction

Carbon dioxide is released into the atmosphere with the burning of fossil fuels, during cement production, and by other human activities (The Royal Society, 2005). Absorption of this anthropogenic CO_2 by the world's oceans has reduced global mean surface water 0.1 pH units below pre-industrial levels, an increase in pH of about 30% (Caldeira and Wickett, 2003). This reduction in ocean's pH due to anthropogenic CO_2 is referred to as ocean acidification. In addition to reducing oceanic pH, surface waters that are currently supersaturated ($\Omega>1$) with respect to calcium carbonate, which is needed to build and maintain calcified structures such as shells, are expected to become undersaturated ($\Omega<1$) (Orr et al., 2005).

High latitude waters are particularly vulnerable to ocean acidification because of a loss of sea ice, high rates of primary productivity, and increased oceanic uptake of anthropogenic CO_2 (Fabry et al., 2009). Additionally, carbonate ion concentrations are naturally low in these waters compared to mid and low latitudes. High latitude waters are predicted to be the first to be persistently undersaturated with respect to calcium carbonate, which could occur within decades not centuries as previously suggested (Orr et al., 2005; Fabry et al., 2009; Mathis et al., 2011b). Persistent undersatuation of calcite in high latitudes is predicted to lag aragonite by 50-100 years (Orr et al., 2005). In the North Pacific, the calcite saturation horizon has already shoaled by approximately 40 to 100 m since pre-industrial times, and the highest dissolution rates of calcium carbonate occur between 400 and 600 m (Feely et al., 2004). The bottom waters inside a bay in the Gulf of Alaska and along the inner shelf are already undersaturated with respect to aragonite for part of the year (Fabry et al., 2009). In the eastern Bering Sea, aragonite is undersaturated in broad regions across the shelf for part of the year (Mathis et al., 2011a), and calcite was undersaturated for at least one month in a few areas over the shelf (Mathis et al., 2011b).

Based upon a meta-data analysis of the effects of ocean acidification on physiological performance of five taxa (corals, echinoderms, mollusks, crustaceans and fishes) crustaceans are less sensitive to ocean acidification than the other invertebrates examined, but many crustacean species will still likely be affected by ocean acidification (Wittmann and Poertner, 2013). Although crustaceans are less sensitive to ocean acidification than other taxa, is has a variety of effects on different life stages of decapods including decreased survival (Kurihara et al., 2008; Long et al., 2013b), decreased growth (Kurihara et al., 2008; Long et al., 2013b), decreased egg production (Kurihara et al., 2008), decreased calcification (Long et al., 2013b; Arnold et al., 2009), increased deformities (Agnalt et al., 2013), increased calcification (Long et

al., 2013a), and increased hatch duration (Long et al., 2013a). Carryover effects of ocean acidification between life stages occur in the crab *Hyas araneus*; larvae hatched and reared in acidified waters from embryos that developed in acidified waters had higher mortality and developmental delays than when just larvae were reared in acidified waters (embryos developed in ambient waters) (Schiffer et al., 2014).

Tanner crab (*Chionoecetes bairdi*) occur in the Pacific Ocean from Oregon to Alaska, in the Bering Sea, Aluetian Islands, and the Sea of Okhotsk (Jadamec et al., 1999) and have historically supported valuable commercial fisheries in Alaskan waters, but in recent years, reduced stock sizes have resulted in many fishery closures (Spalinger, 2013; NPFMC, 2013). Tanner crab are found at depths ranging from subtidal to approximately 437 m with mature male and female Tanner crab migrating to the deeper waters where they remain (Stevens et al., 1993; Jadamec et al., 1999). The deeper depths that Tanner crab are found in are within the range of the highest dissolution rates of calcium carbonate in the North Pacific (Feely et al., 2004). Mating occurs after a female undergoes her molt to maturity which is terminal (final) and in subsequent years she can mate while hard shelled or use stored sperm to fertilize egg clutches (Paul, 1984; Paul and Adams, 1984). Extrusion of a fertilized egg clutch occurs within 48 h of mating, brooding duration is approximately 12 to 16 months and larval hatching typically occurs in April and May (Swiney, 2008; Donaldson and Adams, 1989).

Because Tanner crab are living in waters being affected by ocean acidification, and are of commercial importance, it is critical to examine the potential effects of ocean acidification on different life stages of this species. In this study, the effects of ocean acidification on Tanner crab embryo development, mean number of viable larvae hatched, hatching success, mean hatch duration, mean brooding duration, and calcification rates of adults were determined by rearing ovigerous females in ambient pH, pH 7.8, and pH 7.5 treatments for 2 years.

Methods

Seawater Acidification

Sand filtered seawater was pumped into the laboratory from 15 and 26 m depth intakes in Trident Basin which is approximately 30 m from the laboratory. This seawater was acidified using the same methods described in Long et al. (2013a). In short, a tank of pH 5.5 was established by bubbling CO_2 into ambient seawater. This pH 5.5 water was mixed with ambient seawater in the treatment head tanks via peristaltic pumps controlled by Honeywell controllers and Durafet III pH probes. The ambient head tank did not receive any pH 5.5 water. Waters from the treatment head tanks were then supplied to the individual tubs

females were reared in. pH and temperature were measured daily in each experimental tub using a Durafet III pH probe and when the pH deviated from the target pH by more than ±0.02 pH units the Honeywell controller set points were adjusted to bring the pH back to the target value. Weekly water samples from each treatment were taken, poisoned with mercuric chloride, and sent to an analytic laboratory for dissolved inorganic carbon (DIC) and total alkalinity (TA) analysis.

Sample Collection and Laboratory Study

Ethical approval for this research was not required by any federal, state, or international laws because the study was conducted on invertebrates which are not covered under these laws.

Multiparous female Tanner crab were collected using crab pots and a 3 m beam trawl from Chiniak Bay, Kodiak, Alaska (57°43.25'N, 152°17.5'W) May and June 2011 and brought to the Alaska Fisheries Science Center's Kodiak Laboratory seawater facility in Kodiak, Alaska. Forty-eight healthy females brooding at least a ¾ full clutches of newly extruded eggs with at least one claw and not missing more than three legs total were used in the experiment. All of the females extruded their clutch in the field except for 6 that hatched larvae in the laboratory and after larval hatching were mated with mature males. Females were randomly assigned to one of three acidification treatments based on projected future changes to ocean pH: 1) ambient pH (~8.1), 2) pH 7.8 (c. ~2100), and 3) pH 7.5 (c. ~2200) (Caldeira and Wickett, 2003) for a total of 16 females per treatment. The experiment ran for two years and at the end of the experiment there were 10 females in the ambient treatment, 6 females in pH 7.8 treatment, and 7 females in the pH 7.5 treatment. Each female was reared in a 68 L experimental tub with the treatment water flowing at 1L min^{-1}. Water was chilled and allowed to fluctuate seasonally to ensure appropriate temperatures. Crab were fed a diet of fish and squid to excess twice weekly. However, they were not fed during larval hatching. Females were examined daily to ensure they were alive and pH and temperature were recorded daily for each tub.

Once a month, a small clump of approximately 20 eggs were randomly sampled from each female. The embryo developmental stage was determined using methods described by Swiney (2008) for Tanner crab which was based upon Moriyasu and Lanteigne (1998) methods for staging snow crab, *Chionoecetes opilio*, embryo development. Un-eyed eggs were stained for five minutes with Bouin's solution to facilitate observation of the external morphology of the embryos; eyed eggs were not stained. The stages were determined under a compound microscope at 50x magnification. Embryo developmental stages are provided in Table 1.1. Additionally, digital images of ten fresh eggs from each female were taken with a

digital camera attached to a compound microscope at a total magnification of 63x. Using image analysis software (Image Pro Plus Versions 6.00.260 and 7.0.1.658, Media Cybernetics, Inc., Rockville, Maryland USA), egg area and diameters (maximum, minimum, and average) were measured (Fig. 1.1). Once embryos were discernable, embryo and yolk areas and diameters ((maximum, minimum, and average) were also measured. Lastly, when embryos become eyed, eyespot area and diameters (maximum, minimum, and average) were measured.

In spring 2012 and 2013 larval hatching occurred. Prior to larval hatching, nets were placed on the outflow of each tub to retain all of the larvae from each female and newly hatched larvae were collected and dried daily at 60°C until a constant weight was achieved. Average weight per larvae for each female each year was calculated from approximately 5 replicates of 50 dried larvae. The average weight per larvae for each female was used to calculate how many larvae hatched from the samples dried daily. If less than approximately 200 larvae hatched then all of the larvae were counted. On days when larvae were collected for experiments (see Long et al., in review), the number of viable larvae hatched was estimated by counting the number of larvae in three to four subsamples of known volume and calculating the total number hatched for the day. The number of viable larvae hatched is a little less than what the true number of larvae hatched would have been since we sampled eggs monthly for the embryo development portion of the project, however given that females typically release more than 100,000 larvae, the ~240 eggs removed from each crab is negligible. Toward the end of larval hatching, some larvae did not molt past the pre-zoea stage to the first zoeal stage, and these were deemed to not be viable larvae. During the late stages of larval hatching and prior to extrusion of a new clutch, females clean their pleopods, removing all or nearly all of the empty egg cases and unhatched eggs over the course of several days (Donaldson and Adams, 1989). The debris from each female was collected, examined microscopically and all viable larvae, non-viable larvae, and dead eggs counted in a volumetric subsample. At the end of the experiment, 10% of females in the ambient treatment, 33% of females in the pH 7.8 treatment, and 86% of females in the pH 7.5 treatment did not hatch their entire brood or clean their pleopods. Microscopic examination of these clutches confirmed that they were carrying dead eggs. At the end of the experiment when the females were sacrificed (see below), the abdominal flap with dead eggs attached was removed from these females, frozen, and later processed to estimate the number of dead eggs. Hatching success was defined as the percent viable larvae hatched divided by the calculated total number of larvae that could have hatched (number of viable larvae hatched + number of non-viable larvae hatched + number of eggs that did not hatch). The percent of non-viable larvae hatched and percent of eggs that did not hatch were also calculated. Hatching duration was determined for each female each year with the first day of hatching

386 defined as the first day 50 or more larvae were hatched and hatched ended when females began to strip
387 their pleopods clean. Hatching duration was not determined for the females that did not strip their
388 pleopods clean.
389
390 Females are receptive to mating after cleaning their pleopods (Paul, 1984) so in 2012 mature males were
391 placed in the females' tubs to mate. The females were checked daily and the day that a new clutch was
392 extruded was recorded. It is not known if the females mated or used stored sperm to extrude a new clutch,
393 but all females extruded a viable clutch. Males were not introduced to the females after larval hatching in
394 2013. Brooding duration for each female was defined as beginning the day of egg extrusion in 2012 and
395 ended when larval hatching began in 2013. At the end of the experiment, all females were sacrificed and a
396 portion of their exoskeleton was sent to an analytical laboratory for calcium and magnesium content
397 analysis.
398
399 **Statistical Analysis**
400 The effects of seawater pH on the development of embryos were analyzed using principal component
401 analysis (PCA) in Primer 6.1.15 (Primer-E Ltd, Lutton, UK); each year was analyzed separately. Embryo
402 measurements were normalized prior to analysis. We retained all PCs that were necessary to explain at
403 least 90% of the variation in the data each year. Each retained PC was analyzed with an ANOVA with pH
404 treatment fully crossed with month and female nested in treatment crossed with month as factors. The
405 second PC in the second year failed to meet the assumption of homogeneity of variance so we analyzed
406 the average PC2 score for each female on each date (which met the assumption) using an ANOVA with
407 pH treatment fully crossed with month and female nested in treatment as factors.
408
409 The effects of different seawater pH on mean number of viable larvae hatched, hatching success, mean
410 hatch duration (2012 only), mean brooding duration, and calcification were examined using one-way
411 ANOVAS or Kruskal-Wallis tests with pH as the factor. The 2013 mean hatch duration was examined
412 with a two-sample t-test because hatching duration could only be estimated for one female in the pH 7.5
413 treatment so this treatment was not included in the analysis. Anderson-Darling test for normality and
414 Levene's test for homogeneity of variance were used to determine if data meet the assumptions of
415 ANOVA and when data failed these test the non-parametric Kruskal-Wallis test was used. When
416 significant differences were detected with ANOVAs, Tukey's HSD post-hoc multiple comparisons tests
417 were used to examine the differences between pH treatments. When significant differences were detected
418 with Kruskal-Wallis tests, Conover-Inman tests for all pairwise comparisons were used to examine the

differences between pH treatments. Statistical analyses were conducted in SYSTAT 10.00.05 (Systat Software, Inc. Chicago, Illinois USA).

Results

The mean daily temperature was 5.0 (SD=1.5) °C, varied seasonally from a low of ~1 °C in January 2012 to a high of ~ 9°C in August 2011, and did not vary between treatments (Kruskal-Wallis, H=0.761, P=0.684; Fig. 1.2). Target pHs were achieved throughout the experiment (Table 1.2). As expected, pCO_2 increased with decreasing pH, alkalinity did not vary among treatments, and DIC increased with decreasing pH (Table 1.2). Aragonite was supersaturated in the ambient treatment, but undersaturated in the pH 7.8 and pH 7.5 treatments. Calcite was supersaturated in the ambient and pH 7.8 treatments, and undersaturated in the pH 7.5 treatment (Table 1.2).

Mean embryo stage did not differ among pH treatments for either year one or year two of the experiment (Fig. 1.3). In year one, stage 14, which is the prehatching stage (Table 1.1), was not observed during the monthly sampling because after the April 2012 sampling females were either hatching their larvae or they had finished hatching and extruded a new clutch.

Embryo development was significantly different among pH treatments in both years. PCA results were qualitatively similar between the years. In both years the first two PCs explained more than 90% of the variance and were the only two retained (Tables 1.3 and 1.4). The first PCs were negatively associated with egg, embryo, and eye size and positively associated with yolk size and are interpreted as embryo maturity, with more mature embryos having smaller PC1 scores. The second PCs were positively associated with egg and yolk size. In both years there was a significant interaction between month and treatment on the PC1 scores (Table 1.3 and 1.4, Fig. 1.4); early in development there were no differences among the treatments, but there began to be significant differences starting in December in the first year and in November the second year. These differences persisted though hatching (Table 1.4, Fig. 1.4); the lack of a difference in May in the second year was due to the fact that most of the ambient and pH 7.8 females had already finished hatching at that point. In general, embryos in the ambient and pH 7.8 treatments differed from the embryos in the pH 7.5 treatment, but not from each other. PC2 showed similar trends. In the first year there were significant differences among the pH treatments with the ambient and pH 7.8 treatments differing from the pH 7.5 treatment, but not from each other whereas in the second year this was the case in most, but not all, months leading to a significant interactive effect (Table 1.4, Fig. 1.4). Although there were statistically significant differences among the treatments the

effect size in 2012 was slight. The range in percent difference (positive or negative) between ambient and pH 7.5 embryos for any one morphometric variable was 1.2 % (egg minimum diameter) to 7.1% (eye minimum diameter) while the average was only 3.6% in April 2012 (Table 1.5). In the second year effect sizes were larger; embryos in the pH 7.5 treatment had 10.1% larger yolks and 6.3% smaller embryos than those in the ambient treatment.

The mean number of viable larvae hatched in the first year (2012) of the experiment did not differ with pH treatment (Kruskal-Wallis, H=2.253, P=0.324; Fig. 1.5a). Furthermore, there was not a significant difference in the mean number of viable larvae hatched in the second year of the experiment (2013) among the ambient and pH 7.8 treatments, however on average 71% less viable larvae hatched from the pH 7.5 treatment in 2013 (ANOVA, $F_{2, 27}$=5.796, P=0.008; Fig. 1.5b). For all three treatments, less viable larvae hatched in 2013 than 2012. In the ambient pH treatment on average 47% less viable larvae hatched in 2013 than 2012, in the pH 7.8 treatment 54% less viable larvae hatched in 2013, and in the pH 7.5 treatment 83% less viable larvae hatched in 2013 than 2012 (Fig. 1.5).

Hatching success, measured as the percent of viable larvae hatched, did not differ in 2012 among pH treatments and averaged 99% (Kruskal-Wallis, H=0.265, P=0.876; Fig. 1.6). In 2013 hatching success was on average lower in the pH 7.5 treatment than the ambient treatment and the ambient and pH 7.8 treatments did not differ (Kruskal-Wallis, H=7.988, P=0.018; Fig. 1.6). Overall fewer viable larvae were hatched in 2013 than 2012 as 99% hatching success was observed in 2012 and in 2013 hatching success averaged 46% to 87% depending on the pH treatment (Fig. 1.6). More non-viable larvae hatched in 2013, averaging 3% to 15% depending on pH treatment (Fig. 1.6), in comparison to less than 1% observed in 2012. Likewise, on average more eggs did not hatch in 2013, 10% to 39% depending on pH treatment (Fig. 1.6), in comparison to less than 1% observed in 2012.

Mean larval hatching duration did not differ with pH treatment in either 2012 (Kruskal-Wallis, H=3.783, P=0.151) or 2013 (t-test, t=0.515, p=0.616). In 2012, average larval hatching duration was 6 (S.E =0.182) days and ranged from 4 to 8 days. In 2013, average larval hatching duration was longer and more variable averaging 16 (S.E.=3.537) days and ranged from 6 to 59 days with only one female from treatment pH 7.5 completing hatching. Mean brood duration, which was only estimated for year 2 of the experiment, did not differ with pH treatment (Kruskall-Wallis, H=0.800, P=0.670) and averaged 356 (S.E.=1.150) days.

Adult females from the ambient pH treatment had the highest survival rate (63%) followed by females in the 7.5 pH treatment (44%), and survival was lowest in the 7.8 pH treatment (38%); however the difference in survival between the 7.8 and 7.5 pH treatments was only one crab. The percent dry weight of calcium in the females' carapaces at the end of the experiment was significantly less in the pH 7.5 treatment than either the ambient or pH 7.8 treatments which did not differ (Kruskal-Wallis, H=11.041, P=0.004; Fig. 1.7a). The percent dry weight of magnesium did not differ with pH treatment (ANOVA, $F_{2,16}$=0.929, P=0.415; Fig. 1.7b). The ratio of magnesium and calcium differed between the ambient and 7.5 pH treatments, but did not differ among the other pH treatments (ANOVA, $F_{2,16}$=5.108, P=0.019; Fig. 1.7c). The carapaces of females reared in the pH 7.5 treatment were noticeably more pliable than the females reared in the other pH treatments.

Discussion

Decreased pH had a slight and likely not biologically significant effect on Tanner crab embryo development and no effect on larval hatching in year one of this study; however significant differences were detected in embryo development, the number of viable larvae hatched, and hatching success in year 2. Oocytes that became the embryos and subsequently larvae in year one were developed in the field, whereas for year 2 the oocytes developed under treatment conditions during year one of the study (oocytes that will become embryos the subsequent year develop for approximately one year while the female is brooding a clutch of eggs). In short, embryos and larvae in year 2 of the study were the result of oocytes developed under treatment conditions. The lack of effects with decreased pH in year 1 of the study suggests Tanner crab embryo development and larval hatching is not very sensitive to ocean acidification if oocytes are developed under ambient conditions. Further, observed effects with some parameters in year 2 of the study suggests that Tanner crab are susceptible to the effects of ocean acidification during oocyte development and that these effects carry over into embryo development and larval hatching success. Variability in the sensitivity of different life stages and carry over effects between stages to acidified waters have been found for other crab species. For the great spider crab Hyas *araneus,* larval mortality was significantly higher and developmental delays observed under acidified conditions when larvae were hatched from embryos developed in acidified waters in comparison to larvae exposed to acidified conditions but embryos that did not develop under acidified conditions (Schiffer et al., 2014). For the porcelain crab *Petrolisthes cinctipes*, the embryo stage is more sensitive to acidified waters than the larval and juveniles stages (Carter et al., 2013). These studies highlight the need for ocean acidification research to examine the potential effects of acidification on all stages and for different species (Kurihara, 2008).

Without carry over effects from oocytes developing in acidified conditions, the embryo development phase for Tanner crab appears to be relatively robust to the effects of acidified waters. In year 1 of the study, significant effects were detected between the pH 7.5 treatment and the other 2 treatments, but the effects were minimal and likely not biologically significant. Likewise, acidified waters (reduced pH by 0.4 units) did not affect embryo development rate, heart rate, or oxygen consumption of the Norway lobster *Nephrops norvegicus* (Styf et al., 2013). The oocytes that developed into the embryos in the Norway lobster study developed under ambient conditions, the same as year one of our study. It would be interesting to determine if the Norway lobster oocytes are sensitive to acidification and if there are carry over effects as we observed with Tanner crab. In contrast, the embryo development phase for red king crab *Paralithodes camtschaticus*, the great spider crab, and the porcelain crab appear to be sensitive to acidified waters, even with only short-term exposure (Schiffer et al., 2014; Long et al., 2013a; Carter et al., 2013).

Tanner crab embryos that developed in pH 7.5 waters had larger yolks and smaller embryos than embryos reared in pH 7.8 and ambient waters suggesting that development was delayed for embryos reared in the lowest pH treatment. However, the developmental delay was not large enough to cause a difference in the mean monthly embryo stages among the treatments or brood duration. Similarly, exposure of porcelain crab embryos to acidified waters (pH 7.6) resulted in lower metabolism and dry mass which may cause delayed development (Carter et al., 2013). In contrast, red king crab embryos that developed in acidified waters (pH 7.7) had larger embryos and smaller yolks suggesting an increase in development rate under acidified conditions (Long et al., 2013a).

Acidified waters had a pronounced effect on the reproductive output in year 2 of this study; again suggesting that exposure to acidified waters during oocyte development carry over to later stages. In year one of the study, acidified waters did not have an effect in the number of viable larvae hatched, but in year 2 of the study females reared in pH 7.5 waters hatched on average 71% fewer viable larvae than the other two treatments. Further, acidified waters did not affect hatching success in year one of the study, but in year 2 hatching success in the pH 7.5 treatment was 40% less than in the ambient treatment. This considerable reduction in the reproductive output of females reared in the pH 7.5 waters will likely have a substantial impact on Tanner crab population size and fisheries under these conditions. Likewise, acidified waters (pH 7.89) reduce the reproductive output of the marine shrimp *Palaemon pacificus* by reducing the number of clutches individual females' brood (Kurihara et al., 2008).

For all the treatments, fewer viable larvae hatched and hatching success was lower, in year 2 than year 1 of the study which may have occurred due to senescence and/or laboratory affects. The closely related snow crab *Chionoecetes opilio*, is thought to have a maximum post-terminal molt life span of 6-7 years in the eastern Bering Sea (Ernst et al., 2005), and become senescent with age (Kon et al., 2010). Longevity information on Tanner crab is lacking (NPFMC, 2013), but if Tanner crab are similar to snow crab the reduction in viable larvae hatched and hatching success in year 2 of the experiment may have been due to senescence. The females used in this study were multiparous in year 1 so they were at a minimum 3 years post-terminal molt during year 2 of the study. Regardless of the reason for reduced numbers of viable larvae hatched and hatching success in year 2, we are confident that the differences observed with treatment are real since all of the crab were in the laboratory for the same amount of time so laboratory effects should be the same for all treatments.

Acidified water did not affect Tanner crab larval hatching duration in either year of the study, suggesting that this process is not sensitive to acidification for this species. These results support the notion that hatching duration is innate, heritable, and not easily altered by most environmental parameters (Morgan, 1995). It has been hypothesized that Tanner crab hatching is controlled by an endogenous biological clock and that hatching corresponds to the period of greatest tidal exchange to optimize larval transport (Stevens, 2003). Hatching was longer and more variable for all treatments in year 2 of the study which may have been the result of senescence or laboratory effects. With the crab being in the laboratory for 2 years, their biological clocks may have been affected by the crab not having direct environmental influences such as tidal fluctuations which appear to be important cues for hatching. In contrast, red king crab hatching duration was 33% longer in acidified waters (pH 7.7) (Long et al., 2013a). King crab have a longer hatching duration than Tanner crab and it is thought that the longer hatching duration is an evolutionary response to conditions that were highly variable and unpredictable. A longer hatching duration increases that chance that some larvae will be released during a time of high food availability which will improve larval survival (Stevens, 2006). For red king crab, acidified waters may be perceived as increased environmental variability resulting in the longer hatching duration observed (Long et al., 2013a).

Based upon the few adult decapod species examined, acidified waters have either a positive or no effect on calcification (Landes and Zimmer, 2012; Ries et al., 2009; Long et al., 2013a). In this study, the reduced calcification (percent dry weight of calcium) in the 7.5 pH treatment is unprecedented. The difference between our results and other studies may simply be that in our experiment the crab were

reared in acidified water for 2 years whereas the previous studies were months (maximum of 5 months) in duration. Alternately, Tanner crab physiological responses to acidification may differ from other decapod species as was observed in a long term study (nearly 200 days) comparing the responses of juvenile Tanner and red king crab to ocean acidification. Juvenile Tanner crab had decreased calcium content and an unaffected condition index, whereas juvenile red king crab calcium content was unaffected but the condition index was reduced (Long et al., 2013b). Ries et al. (2009) suggests that organisms (i.e. decapods) that have unaffected or increased calcification under acidified conditions elevate pH at the site of calcification and depending upon the efficiency of their specific proton-regulating mechanism end up with similar conditions at the site of calcification as they currently experience. It is unclear why Tanner crab have reduced calcium content under acidified conditions, but they may not be as efficient at increasing pH at the site of calcification as other decapods. Alternatively, the calcium carbonate in the carapace may be dissolved internally in an attempt to buffer internal pH decreases. Long term ocean acidification studies are needed to see if other decapod species experience reduced calcification or if this response is unique to Tanner crab.

Projected ocean pH levels within the next 2 centuries will likely have a pronounced negative effect on Tanner crab populations unless the crab are able to adapt to rapidly changing conditions. Tanner crab appear to be resilient to medium term (1 year) exposure to acidified waters. However, the distressing effects of ocean acidification on Tanner crab populations exposed to acidified water for the entire reproductive cycle (from oocyte development to hatching) is apparent by the relatively small number of viable larvae hatched among females reared in the pH 7.5 treatment year 2 of this study. Further, calcification was reduced among females reared in the pH 7.5 treatment and their carapaces were noticeably more pliable than females reared in the other treatments. Results from this study highlight the need for long term ocean acidification research that encompasses many life stages so potential carry over effects can be observed.

Acknowledgements

This project was funded by the North Pacific Research Board (NPRB publication no. XXX) and National Marine Fisheries Service (NMFS). We thank NMFS biologists and laboratory technicians for their assistance which made this project possible. Previous versions of this paper were improved by comments from XXXXXXXX. The findings and conclusions in the paper are those of the authors and do not necessarily represent the views of the National Marine Fisheries Service, NOAA. Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

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698

699 Table 1.1 Embryo developmental stages based upon Moriyasu and Lanteigne (1998) descriptions for
700 *Chionoecetes opilio.* See Moriyasu and Lanteigne (1998) for detailed descriptions.

Stage #	Embryo Developmental Stage
1	Prefuniculus formation
2	Funiculus formation
3	Cleave and blastula
4	Gastrula
5	Lateral ectodermal band
6	Prenauplius
7	Nauplius
8	Maxilliped formation
9	Metanauplius
10	Late metanauplius
11	Eye-pigment formation
12	Chromotophore formation
13	Reduced yolk
14	Prehatching

701

Table 1.2. The mean and standard deviation (SD) of water chemistry parameters in the three treatments during the experiments. pH was measured daily, DIC and alkalinity were measured weekly, and all other parameters were calculated.

Treatment	pH	pCO_2 µatm	HCO_3^- mmol/kg	CO_3^{-2} mmol/kg	DIC mmol/kg	Alkalinity mmol/kg	$\Omega_{Aragonite}$	$\Omega_{Calcite}$
Ambient	8.09(0.07)	391.90(65.59)	1.90(0.04)	0.09(0.02)	2.01(0.04)	2.13(0.06)	1.44(0.25)	2.31(0.40)
pH 7.8	7.80(0.03)	781.17(31.13)	1.99(0.04)	0.05(0.00)	2.08(0.04)	2.13(0.06)	0.78(0.05)	1.24(0.07)
pH 7.5	7.50(0.03)	1597.15(62.76)	2.05(0.04)	0.03(0.00)	2.16(0.04)	2.13(0.04)	0.40(0.02)	0.64(0.04)

Table 1.3: Principal component analysis of Tanner crab embryo morphometrics in the first year. The first two principal components (PCs), representing 93% of the cumulative variation, are retained. ANOVA analysis with treatment (T) fully crossed with month (M), and crab (nested) was performed for the first two eigenvectors.

PC	Eigenvalues	%Variation	Cum.%Variation
1	10.8	82.8	82.8
2	1.32	10.2	92.9

	Eigenvectors	
Variable	**PC1**	**PC2**
Egg Area	-0.273	0.377
Egg Max Diameter	-0.231	0.45
Egg Min Diameter	-0.269	0.275
Egg Average Diameter	-0.272	0.378
Yolk Area	0.276	0.334
Yolk Max Diameter	0.256	0.365
Yolk Min Diameter	0.268	0.282
Yolk Average Diameter	0.277	0.327
Embryo Area	-0.301	-0.06
Eye Area	-0.291	0.025
Eye Max Diameter	-0.29	-0.039
Eye Min Diameter	-0.297	-0.01
Eye Average Diameter	-0.297	-0.024

ANOVA			
Variable	**Factor**	**F**	**p**
PC1	Treatment	77.291	<0.0005
	Month	21,702.58	<0.0005
	T*M	10.025	<0.0005
	Crab(T*M)	9.027	<0.0005
PC2	Treatment	12.355	<0.0005
	Month	28.304	<0.0005
	T*M	0.931	0.548
	Crab(T)	10.286	<0.0005

711 Table 1.4: Principal component analysis of Tanner crab embryo morphometrics in the second year. The
712 first two principal components (PCs), representing 94% of the cumulative variation, are retained.
713 ANOVA analysis with treatment (T) fully crossed with month (M), and crab (nested within treatment
714 crossed with month) was performed for the first four eigenvectors.

PC	**Eigenvalues**	**%Variation**	**Cum.%Variation**
1	10.5	80.6	80.6
2	1.73	13.3	93.9
	Eigenvectors		
Variable	**PC1**	**PC2**	
Egg Area	-0.261	0.398	
Egg Max Diameter	-0.231	0.429	
Egg Min Diameter	-0.256	0.331	
Egg Average Diameter	-0.259	0.405	
Yolk Area	0.284	0.271	
Yolk Max Diameter	0.249	0.367	
Yolk Min Diameter	0.282	0.254	
Yolk Average Diameter	0.275	0.313	
Embryo Area	-0.305	-0.084	
Eye Area	-0.299	-0.050	
Eye Max Diameter	-0.291	-0.013	
Eye Min Diameter	-0.302	-0.045	
Eye Average Diameter	-0.300	-0.030	

ANOVA			
Variable	**Factor**	**F**	**p**
PC1	Treatment	104.60	<0.0005
	Month	16,523.18	<0.0005
	T*M	5.318	<0.0005
	Crab(T*M)	13.807	<0.0005
PC2	Treatment	121.16	<0.0005
	Month	297.588	<0.0005
	T*M	5.607	<0.0005
	Crab(T*M)	11.576	<0.0005

715 Table 1.5. The average morphometric measurements measured for each treatment and control in 2012 and 2013. (Dia.=Diameter)

Treatment	Egg Area mm^2	Egg Max Dia. mm	Egg Min Dia. mm	Egg Average Dia. mm	Yolk Area mm^2	Yolk Max Dia. mm	Yolk Min Dia. mm	Yolk Average Dia. mm	Embryo Area mm^2	Eye Area mm^2	Eye Max Dia. mm	Eye Min Dia. mm	Eye Average Dia. mm
2012													
Control	0.293	0.632	0.590	0.610	0.069	0.371	0.227	0.289	0.223	0.028	0.234	0.149	0.186
7.8	0.290	0.628	0.588	0.607	0.074	0.378	0.239	0.300	0.216	0.027	0.231	0.144	0.182
7.5	0.284	0.619	0.583	0.600	0.073	0.379	0.234	0.298	0.211	0.026	0.231	0.138	0.178
2013													
Control	0.284	0.620	0.582	0.600	0.041	0.251	0.158	0.196	0.244	0.029	0.229	0.156	0.188
pH 7.5	0.273	0.606	0.572	0.588	0.045	0.276	0.160	0.208	0.228	0.028	0.232	0.148	0.185
pH 7.8	0.290	0.625	0.589	0.606	0.051	0.288	0.189	0.232	0.239	0.030	0.235	0.157	0.191

716

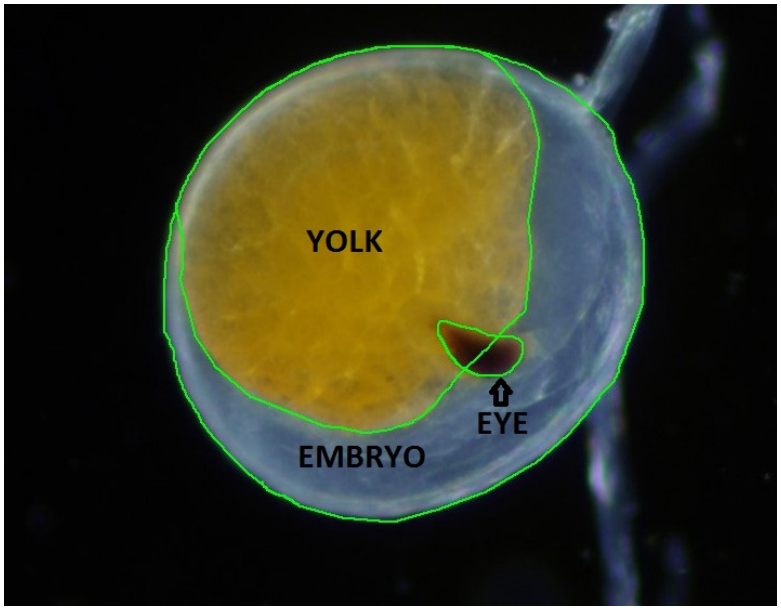
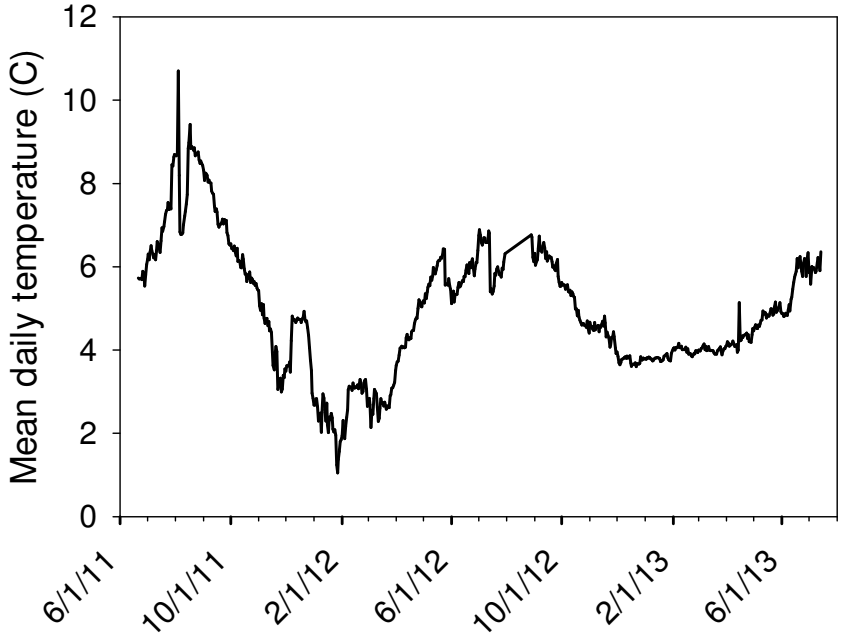


Figure 1.1. Picture of egg with yolk, embryo, and eye area measurements.



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721 Figure 1.2. Mean daily temperature (°C) for the duration of the experiment.

722

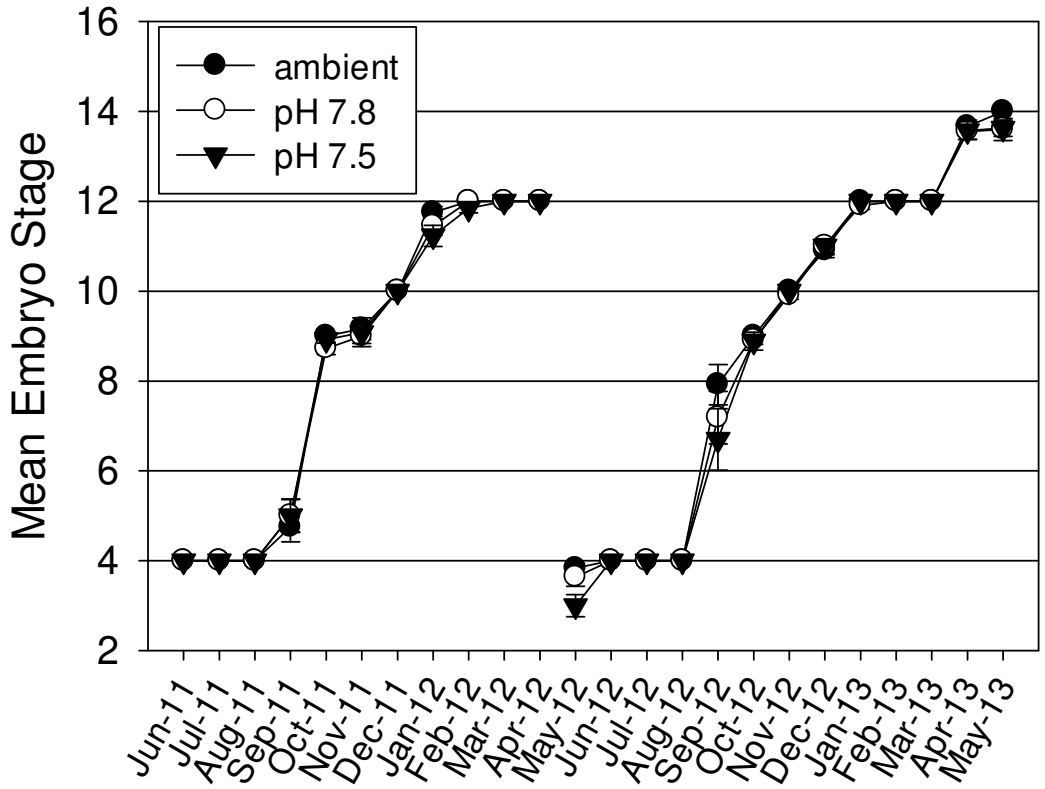


Figure 1.3. Comparison of Tanner crab mean embryo stage by pH treatment for year 1 and 2 of the experiment. See table 1 for embryo stage descriptions. Values are means and vertical bars represent standard errors.

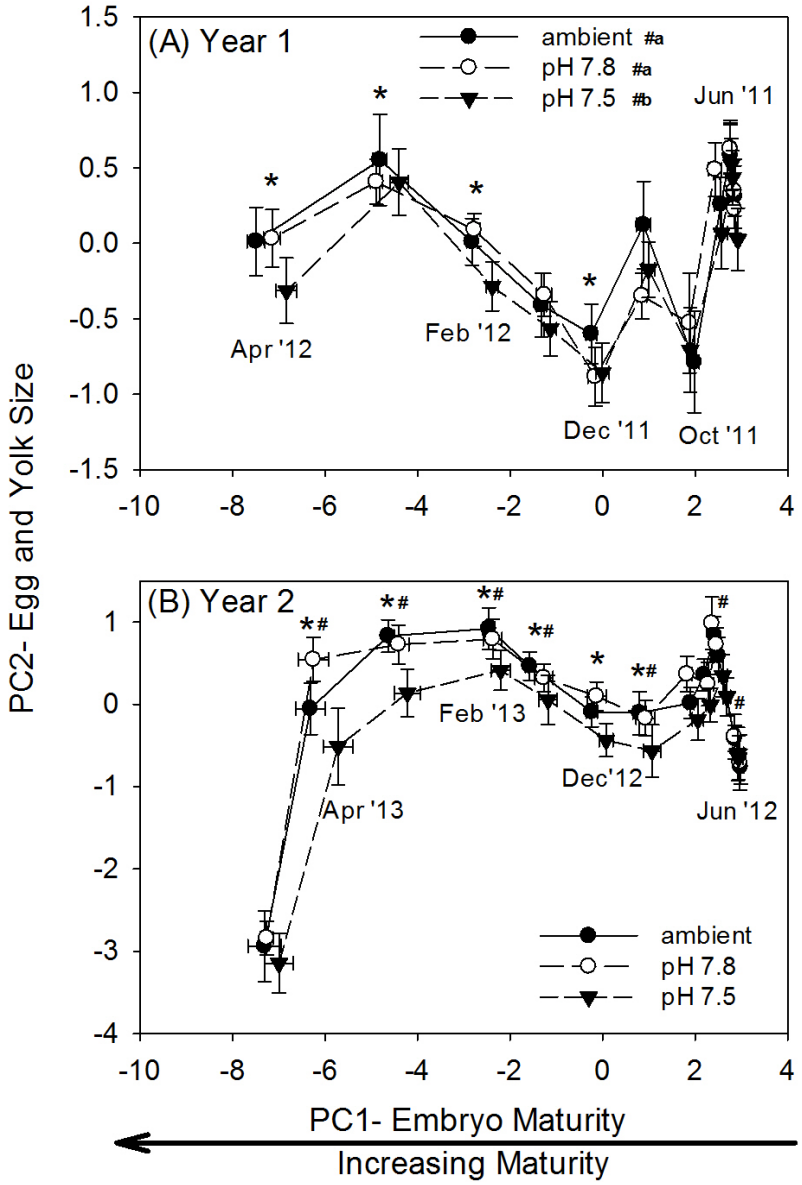
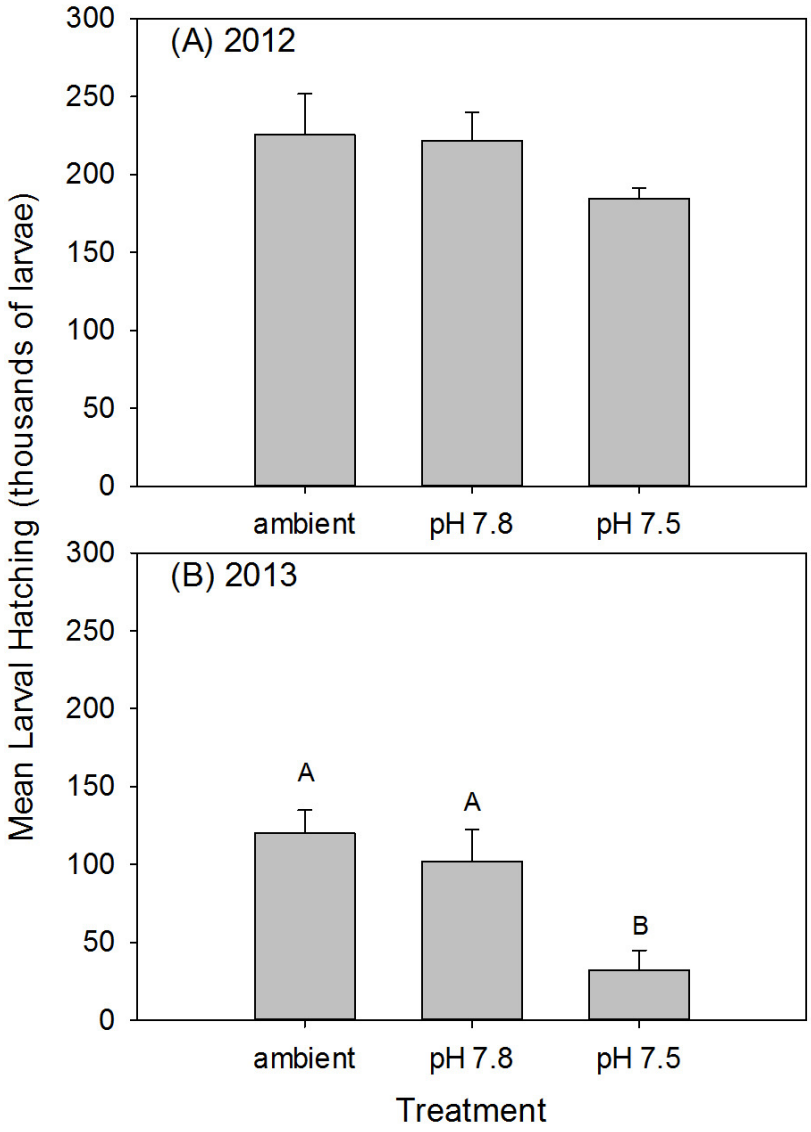
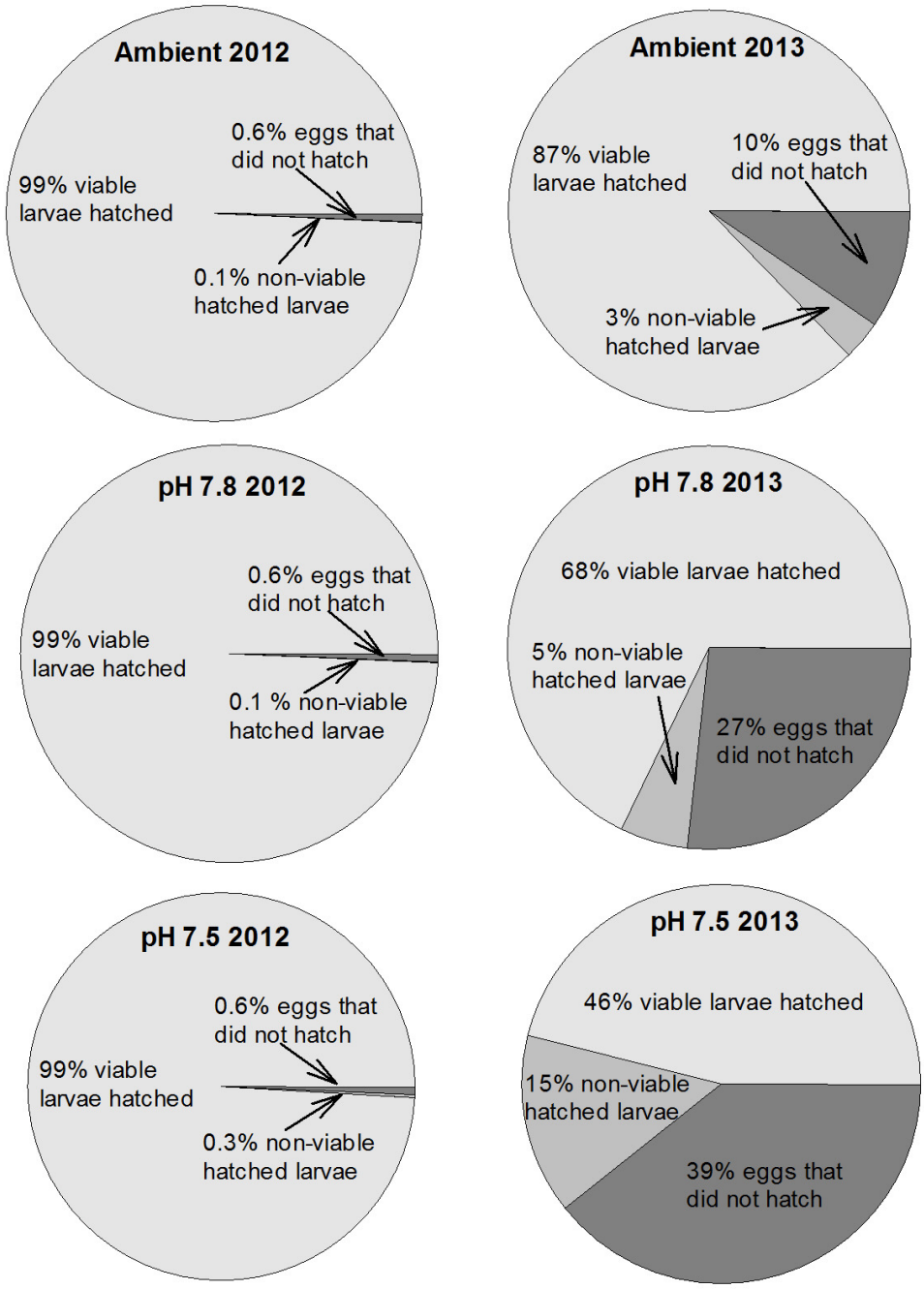


Figure 1.4. Average principal component (PC) 1 and 2 scores ± standard errors for Tanner crab embryos measured monthly during the (A) first and (B) second years of brooding. PC1 is associated with embryo maturity and PC2 is associated with egg and embryo size. Every other month is labeled. Where there were significant differences among treatments within a month it is indicated with * for PC1 and a # for PC2. Where there were differences among the treatments in all months, it is indicated in the figure legend using the same symbols; treatments with the different letters next to them are significantly different.



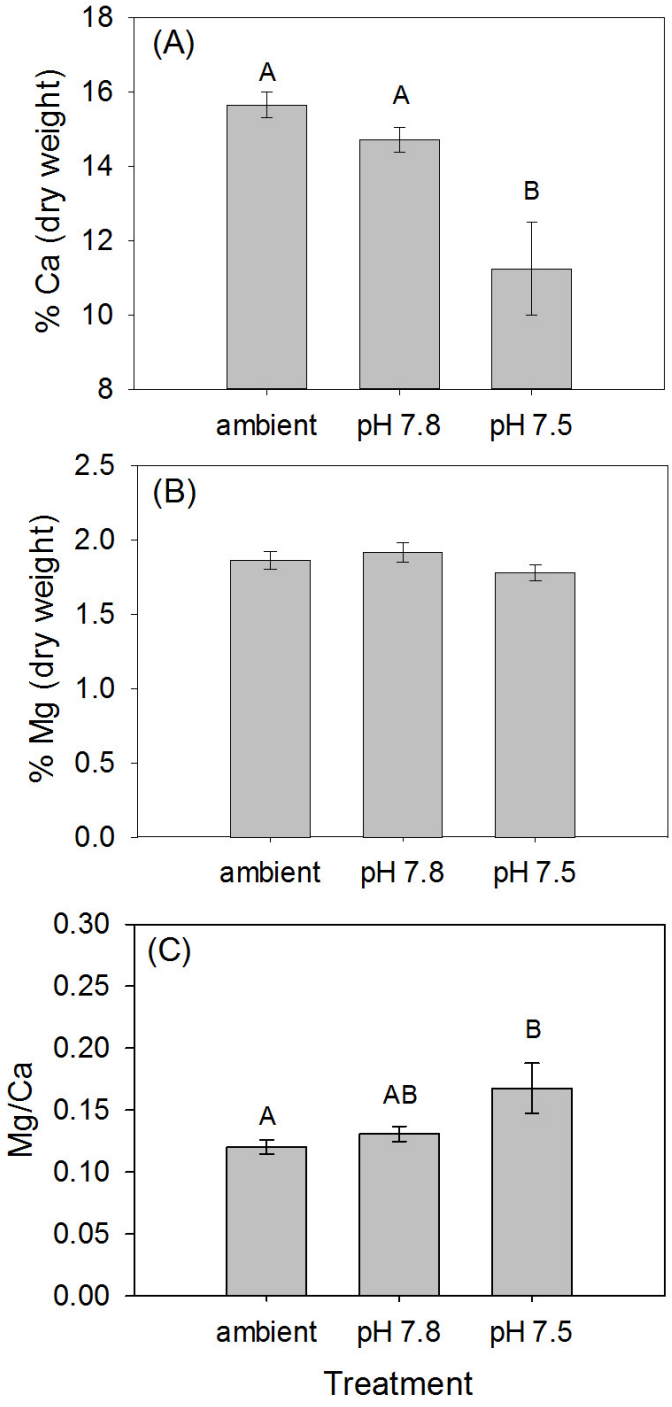
738

739 Figure 1.5. Comparison of the mean number of viable Tanner crab larvae hatched by pH treatment in (A)
740 2012 and (B) 2013. Bars are mean with standard error. Bars with different letters above them differ
741 significantly.



742

743 Figure 1.6. Comparison of the mean percent viable Tanner crab larvae hatched, non-viable larvae hatched,
744 and eggs that did not hatch in 2012 and 2013 among ambient, pH 7.8, and pH 7.5 treatments.



745

746 Figure 1.7. Comparison of the mean (A) percent dry weight calcium, (B) percent dry weight magnesium,
747 and (C) magnesium calcium ratio from carapaces of females reared in ambient, 7.8 pH and 7.5 pH
748 treatments for two years. Bars are mean with standard error. Bars with different letters above them
749 differ significantly.

750

Manuscript #2: Effects of ocean acidification on Tanner crab larvae: Carryover effects from oogenesis and embryogenesis are stronger than direct effects.

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Abstract:

Anthropogenic CO_2 release is increasing the CO_2 concentrations in the atmosphere and oceans and causing a decrease in the pH of the oceans. This decrease in pH, known as ocean acidification, can have substantial negative effects on ocean animals. In this study we examine the effects of ocean acidification on the larvae of the economically important Tanner crab, *Chionoecetes bairdi*. By using wild-brooded larvae and holding females in treatment pH for two brooding cycles over two years and using the resulting larvae, we were able to detect carryover effects from oogenesis and embryogenesis. Ovigerous females were held at three pHs, ~8.2 (Ambient), 7.8, and 7.5. When larvae hatched, they were used in experiments examining the effects of ocean acidification on starvation-survival, morphology, condition, and calcium/magnesium content. Exposure to acidified conditions at the larval stage alone had minimal effects on the larvae, likely because larvae are adapted to living in an environment with large pH swings. However, exposure during oogenesis and embryogenesis resulted in significant carryover effects; larvae differed morphometrically, were smaller, and had lower calcium and magnesium contents. They also had a lower metabolic rate as evidenced by longer starvation-survival times. Although the larval phase itself is resilient to low pH, carryover effects are likely to have a negative effect on larvae in the wild. These results, combined with negative effects of ocean acidification at other life history stages indicate that ocean acidification may have a significant effect on the Tanner crab populations and fisheries in the near future.

Keywords: Ocean acidification, hypercapnia, crabs, Tanner crabs, mortality, early life history

Introduction

783 Anthropogenic activities are causing an increase in the concentration of CO_2 in the atmosphere and a
784 commensurate increase in the oceans (Caldeira and Wickett, 2003; Orr et al., 2005). As the CO_2 in the
785 oceans increases, it is changing the carbonate chemistry of the water, decreasing the pH and increasing
786 the solubility of calcium carbonate. The predicted drop in pH in the oceans, about 0.3 units by 2100 and
787 0.5 units by 2200, is known as ocean acidification (Caldeira and Wickett, 2003). Although ocean
788 acidification is a global phenomenon, it will not affect all areas equally or at the same rate. For example,
789 high latitude areas are expected to be affected sooner than other areas because of the higher solubility of
790 CO_2 in colder water (Fabry et al., 2009). Ocean acidification is expected to have a physiological effect on
791 many marine animals, particularly on those with calcium carbonate shells or exoskeletons (Fabry et al.,
792 2008).
793
794 Although crustaceans are among the calcifying marine organisms, their response to ocean acidification is
795 mixed (Kroeker et al., 2013; Wittmann and Portner, 2013). Some species have reduced survival (Walther
796 et al., 2010; Long et al., 2013b) and growth (Kurihara et al., 2008; Keppel, 2012), but others are resilient
797 to such changes (Pansch et al., 2013; Styf et al., 2013). That crustaceans calcify via an internal process (as
798 opposed to most mollusks, for example) and that the calcified portion of their exoskeletons is protected by
799 a layer of organic matrix may explain some of the mixed results (Ries et al., 2009); increased acidification
800 may cause calcification to increase (Ries et al., 2009; Long et al., 2013a) or decrease (Walther et al.,
801 2011; Long et al., 2013b), or have no effect (Long et al., 2013b). The direction of the response may be
802 due to differences among species' ability to maintain a constant internal pH (Pane and Barry, 2007;
803 Spicer et al., 2007; Hammer and Pedersen, 2013).
804
805 The Tanner crab, *Chionoecetes bairdi*, is a commercially important crab species in Alaskan waters
806 (NPFMC, 2011). Primiparous (brooding first egg clutch) Tanner crab females molt to maturity, mate, and
807 extrude a batch of eggs in the late winter (Paul and Adams, 1984), whereas multiparous (brooding a
808 second or subsequent egg clutch) females release their larvae, mate, and extrude a new clutch of eggs in
809 the late spring (Munk et al., 1996). Eggs are brooded for about a year prior to larval hatching (Swiney,
810 2008); during that year oogenesis is occurring in the female ovaries preparing oocytes for the next years
811 clutch. The larvae spend 2-3 months in the plankton as they pass through a prezoeal stage, 2 zoeal stages,
812 and a megalops stage prior to settling into the benthic environment and molting to the first crab stage
813 (Incze et al., 1982). After passing through successive molts, Tanner crabs likely reach maturity at about 6
814 years (Donaldson et al., 1981). Because of the high latitudes at which Tanner crabs live, the rate of
815 acidification is likely to be high for this species. There is evidence that seasonal variability in the pH of

816 bottom water in Bering Sea results in pH levels that are physiologically stressful to juvenile Tanner crabs
817 for at least part of the year (Mathis et al., 2011; Cross et al., 2013). Juvenile Tanner crabs exhibit
818 decreased growth, survival, and calcification under near-future levels of CO_2 (Long et al., 2013b). Given
819 the sensitivity of this species to ocean acidification it is imperative to understand its effects at all life-
820 history stage. In this paper, we examine the effects of ocean acidification on the survival, condition, and
821 calcium/magnesium content of larval Tanner crabs. In addition, we examine carryover effects to the larval
822 stage from exposure during oogenesis and embryogenesis.
823
824 **Methods**
825 **Overview**
826 To determine the effects of ocean acidification on the larval stage of Tanner crabs, we performed three
827 sets of experiments over three years, 2011-2013. In the first year, 2011, we used larvae that had been
828 brooded in the wild to determine the effects on the larval stage only. We then held females with newly
829 extruded embryos in the laboratory at three pH treatments, ambient and two experimental, until the larvae
830 hatched. In the second year, 2012, we used these larvae, the embryos of which had developed under
831 treatment conditions, to determine carryover effects of exposure at the embryo stage on larvae. Finally,
832 we held the females for another year in their pH treatments. So, in the third year, 2013, the larvae that
833 hatched out came from oocytes that developed in the females while she was in treatment water and
834 embryos that had developed in treatment water. These larvae allowed us to determine carryover effects of
835 exposure during both oogenesis and embryogenesis on larvae.
836
837 **Water acidification**
838 Water was acidified using the same methods as Long et al. (2013a). Incoming filtered seawater at ambient
839 conditions was acidified to pH of 5.5 by bubbling CO_2 into it. This water was mixed with filtered ambient
840 seawater using a peristaltic pump into two head tanks to reduce the pHs to 7.8 and 7.5 (expected pHs in
841 ~2100 and 2200). The flow of pH 5.5 water into each tank was controlled by a Honeywell controller with
842 a Duafet II or III pH probe in each of the head tanks. A third head tank (Ambient) did not receive any pH
843 5.5 water. pH and temperature were measured daily using a Durafet II or III pH probe during all
844 experiments. When the pH in the experimental units deviated from the nominal pH by more than 0.02 pH
845 units, the Honeywell controller was adjusted to bring the pH back to the nominal value. Water samples
846 were taken once a week, poisoned with mercuric chloride, and sent to the NOAA Auke Bay lab (2011 and
847 2012) or to the University of Alaska Fairbanks (2013) for analysis of dissolved inorganic carbon and

alkalinity. The seacarb package in R (V2.14.0, Vienna, Austria) was used to calculate the other parameters of the carbonate system (Lavigne and Gattuse, 2012).

2011 wild brooded larvae

Ethical approval for this research was not required by any federal, state, or international laws because the study was conducted on invertebrates which are not covered under these laws. Healthy, multiparous, ovigerous female Tanner crabs were captured in Chiniak Bay, Kodiak, Alaska, in the spring of 2011 in crab pots and a 3 m beam trawl. Four healthy females with clutches of eyed eggs, average carapace width (CW) ± 1 standard deviation (SD) was 93.3 ± 3.1 mm, were used. Females were held in individual tubs with flow-through ambient water, and larvae for the experiments were collected in a single day in water baths and pooled. The larvae were held at ambient pH and temperature for 24 hours prior to beginning the experiments, and only healthy, actively swimming larvae were used. Experiments were performed in PVC inserts with mesh bottoms placed inside beakers, with the tops of beakers covered with a piece of plastic bubble wrap to reduce gas exchange with the atmosphere (Long et al., 2013a). The beakers were kept in a cold room at 5 ºC, and water was changed once a day by moving each insert from its old beaker to a beaker of new treatment water. The pH and temperature were measured in each insert each day. Two experiments were performed to determine the effects of ocean acidification on the larvae: 1) to determine the effects on larval morphology, 2) to determine the effects on larval survival. For each experiment, there were five replicates of each treatment (Ambient, pH 7.8, and pH 7.5).

The larval morphology experiments were performed in 2 L beakers. Each beaker was stocked with approximately 200 larvae. Larvae were measured by photographing them under a stereo microscope and using Image-Pro Plus v. 6.00.260 (Media Cybernetics, Inc., Bethesda, Maryland, USA) to measure the carapace width (including spines), lateral spine length, dorsal spine length, rostro-dorsal length, rostral spine length, and protopodite length as per Webb et al. (2006). Seven larvae were measured initially, prior to the beginning of the experiment. The larvae were held for 10 days and then three larvae from each beaker were measured. Differences in larval morphometrics were analyzed using an analysis of similarity (ANOSIM) performed on a Brey-Curtis similarity matrix with treatment as the factor and visualized using principle component analysis (PCA) in Primer 6.1.13 (Primer, UK).

The larval starvation-survival experiments were performed in 1 L beakers. Each beaker was stocked with approximately 20 larvae. The larvae were checked daily and any larvae that failed to move within a 15

second observation period were considered dead and were discarded. The experiment continued until all larvae were dead. The survival data was fit to a logistic regression model:

$P_m = \frac{1}{1+\left(\frac{t}{t_{50}}\right)^s}$, were P_m is the probability of mortality, t is the time in days, t_{50} is the time of 50% mortality, and s is a slope parameter assuming a binomial distribution of data. We constructed a series of models in which t_{50} or s were allowed to vary among the pH treatment in R 2.14.0 (Vienna, Austria). Post hoc, we noticed that pH 7.8 and Ambient had similar t_{50} values, so we included models in which those two treatments differed from the pH 7.5 treatment. We selected the best model using the Akaike's information criterion corrected for small sample size (AIC_c). Models whose AIC_c differed by less than 2 were considered to explain the data equally well.

2012 and 2013 laboratory reared larvae

Healthy, multiparous, ovigerous female Tanner crabs, CW 98.7 ± 4.8 mm (SD), were captured in Chiniak Bay, Kodiak, Alaska, in the spring of 2011 in crab pots and a 3 m beam trawl. Each female was held in an individual container with flow-through water. A total of 48 females were used and each was randomly assigned to one of the three pH treatments, for a total of 16 replicates per treatment. Details of the female holding conditions and the effects of ocean acidification on embryo development and Tanner crab fecundity and hatching success are published separately (Swiney et al., in review). In short, the females were held during brooding for the first year. At hatching in 2012, the larvae were collected and used for the 2012 larval experiments. After hatching, a male crab was placed in with each of the females to allow for mating, and each female extruded a new batch of eggs. The females were held in their treatment pH for a second year until the embryos hatched out in 2013 and were used for the 2013 larval experiments.

In 2012 and 2013, experiments were performed in the same sized 1 and 2 L PVC inserts as in 2011. These were placed in a tank with flow-through water at the appropriate treatment pH and ambient temperature and the water within the tank was recirculated into each of the inserts. Larvae for the experiments were collected as above. We pooled the larvae from multiple females within each treatment group to begin the experiments. We performed four experiments to determine the effects of ocean acidification on 1) larval survival, 2) larval carbon and nitrogen content (CN), 3) larval calcium and magnesium content, and 4) larval mass. Each of the experiments was fully crossed between embryo treatment and larval treatment, allowing us to examine carryover effects between the stages (Long et al., 2013a), and five replicates were performed for each experiment for each embryo-larval treatment combination. In 2013, however, so few larvae hatched from females in the pH 7.5 treatment (Swiney et al., in review) that we were only able to

perform the starvation survival experiments on these larvae. Prior to beginning the experiments, 5 samples for CN, calcium/magnesium content, and larval mass analyses were taken from the starting pool of larvae. In addition, because we did not observe any change in larval morphology after rearing larvae in acidified water in 2011 (see results below), we did not examine larval morphology after exposure to acidified waters in 2012 or 2013. Instead in 2012 and 2013, we examined if embryos developed under acidified conditions affected larval morphology by assessing 15 newly hatched larvae from each treatment by measuring and analyzing them as above.

The starvation survival experiments were performed in 1 L sized PVC inserts as above until all larvae had died. The data was fit to a series of logistic regressions as above, where the t_{50} or s parameters were allowed to vary linearly with the embryo and larval treatments. The AIC_c was calculated for each model and the best model selected. For the CN, calcium/magnesium content, and larval mass experiments, ~300 larvae were stocked in 2 L sized PVC inserts and held at their treatment pH for 7 days, dried to a constant mass, and then sent to analytical laboratories to be analyzed for CN, calcium, and magnesium contents. A subset of 50 larvae was counted, dried, and massed from both the CN and calcium/magnesium content experiments in 2012, and from the calcium/magnesium experiments only in 2013. The average mass of each larva was calculated. The percent dry mass of carbon, nitrogen, calcium, magnesium, the C:N and Ca:Mg ratios, and the average mass of a larvae were calculated and analyzed with fully crossed 2-way analysis of variance with embryo and larval treatments as factors. In all cases, the assumption of homogeneity of variance was checked with Levene's test and the data transformed to meet the assumption if it did not. When there were significant effects, Tukey's post hoc test was used to detect differences.

Results

Water chemistry

The pH in the larval experiments stayed well within the nominal range in all three years (Table 2.1). Temperatures were slightly (~0.4°C) lower in 2012 than in 2013 but substantially warmer in 2012 and 2013 than in 2011. While the effects of the lower temperatures in 2011 did not show any major changes in the experimental results relative to the controls, there may have been effects on the relative effect such as slowing the mortality rate during the starvation experiments. As expected, pCO_2 increased with decreasing pH. Ambient water was always above saturation for both aragonite and calcite, pH 7.8 water was undersaturated with respect to aragonite, and pH 7.5 water was undersaturated with respect to both aragonite and calcite. Water chemistry parameters in the female treatments, presented elsewhere (Swiney et al., in review), were similar to the conditions in these larval experiments.

2011 wild brooded larvae

There was no effect of treatment on larval morphometrics (Global R = 0.009, p = 0.364); there were no differences among the larvae held in their treatment water for 10 days, and larvae that had been held for 10 days did not differ in morphology from those measured immediately after hatching (Fig. 2.1). There were two models that fit the survival data equally well: In the first, the slope parameter varied among the treatments but there was no difference in the t_{50}, and in the second, the slope parameter varied among the treatments and the t_{50} was the same in Ambient and pH 7.8 larvae, both of which differed from the pH 7.5 larvae (Table 2.2). However, the differences among the treatment were slight, with the t_{50} parameter varying by only 0.18 d, and likely not biologically significant (Table 2.2, Fig. 2.2).

2012 and 2013 laboratory reared larvae

In 2012, there was a statistically significant effect of embryo treatment on larval morphometrics (Global R = 0.074, p = 0.02); however, the low Global R value indicates that the differences are not biologically significant (Clarke and Warwick, 2001). Pairwise comparisons showed the only difference was between pH 7.8 and pH 7.5, and ordination shows that, on average, pH 7.5 were slightly larger than pH 7.8; however, there was significant overlap (Fig. 2.3). In 2013, larval morphometrics differed both statistically and significantly (Global R = 0.383, p < 0.0001), with pH 7.8 and Ambient larvae being larger than pH 7.5 larvae (Fig. 2.4).

In 2012 and 2013, the t_{50} and s parameters varied among treatments at both the embryo and larval stages as well as in their interaction in the best fit models of larval survival (Table 2.3). In 2012, the full model contained a number of parameters whose 95% confidence intervals, as indicated by the standard error of the parameter estimates, substantially overlapped 0. We thus created a post-hoc model in which these parameters were eliminated, and this increased the parsimony of the model substantially (Table 2.3). In both years, the effect of treatment at the embryo stage was larger than treatment at the larval stage; larvae from embryos that developed in pH 7.5 water survived about 3 days longer than those that developed in Ambient water (Fig. 2.5, Table 2.4). However, in 2012 larvae from embryos that had developed in pH 7.8 water were similar to Ambient larvae whereas in 2013 they were intermediate between the Ambient and pH 7.5 larvae. The effect of treatment at the larval stage and the interactive effects were much smaller and no clear pattern in the data was apparent (Fig. 2.5, Table 2.4).

The C and N content of larvae varied with the larval and embryo treatments in both years (Fig. 2.6, Table 2.5). In general, exposure to acidified water at the embryo and larval stages increased the C and N content of the larvae, with the effect of the embryo treatment being larger (Fig. 2.6). Larvae exposed to pH 7.8 as embryos did not differ from the Ambient larvae in 2012, but did in 2013. In addition, there was always a drop in the C and N content between the initial measurement made right after hatching and after 7 days of larvae starvation. Although there was a significant effect of treatment on the C:N ratio in 2013 (though not in 2012), the effect was small and no pattern is discernable (Fig. 2.6, Table 2.5).

Larval Ca and Mg content varied with the larval and embryo treatments in both years (Fig. 2.7, Table 2.5). Exposure to acidified water at the embryo stage reduced the Ca content in both years and the Mg content in 2012 (the lack of a difference in 2013 is probably driven by the fact that we were unable to run this experiment with pH 7.5 larvae). Ca content was highest in larval reared at pH 7.8 and was lowest in those reared at pH 7.5, with those reared at the ambient in between. Larvae also generally increased their Ca and Mg contents during their first 7 days as larvae (Fig. 2.7). In 2012, the Mg content of larvae exposed to pH 7.5 water at the embryo stage was lower than the other two treatments. The Ca:Mg ratio also decreased in acidified water at the embryo stage, and was higher in larvae reared at pH 7.8 during the larval phase (Fig. 2.7). In order to meet the assumption of homogeneity of variance, the Ca:Mg ratio in 2012 was square root transformed, and the 2013 Mg content was log transformed prior to analysis.

In 2012, the average larval mass varied among the larval treatments, with those reared in pH 7.8 being highest, pH 7.5 being intermediate, and ambient being lowest (Fig. 2.8, Table 2.5). In 2013, larval masses varied among embryo and larval treatments. Ambient water at both the embryo and larval stages was associated with higher larval mass (Fig. 2.8).

Discussion

Exposure to acidified water had a significant effect on the larvae of Tanner crabs, and there were significant carryover effects from exposure of the females during oogenesis and embryogenesis. Although there were no biologically significant effects on wild brooded larvae on survival time, exposure to acidified water at the embryo stages increased survival time under starvation conditions, but was associated with smaller, less calcified larvae. Although there were differences between the ambient and pH 7.8 treatments, biologically significant differences were mostly apparent at pH 7.5, indicating that Tanner crabs are moderately sensitive to ocean acidification. However, given the presence of carryover effects and the effects ocean acidification has on other life history stages, our results indicate that ocean

1010 acidification may have a substantial effect on Tanner crab populations, and therefore fisheries, within the
1011 next 80 years.
1012
1013 Exposure to acidified water at only the larval phase had little effect on larvae. We saw no biologically
1014 significant differences among larval treatments for the wild brooded larvae, and only slight (if any)
1015 differences in carbon and nitrogen content and calcium or magnesium content in the lab-brooded larvae.
1016 Effects were larger in 2013, but the biggest difference was lower calcium content followed by higher
1017 percent carbon and nitrogen contents in pH 7.5 larvae. However, because in 2013 the pH 7.5 larvae also
1018 had a smaller average mass, the total carbon and nitrogen content per larvae were similar among
1019 treatments despite higher percent carbon and nitrogen. Of the life history stages tested to date, including
1020 the embryo (Swiney et al., in review) and juvenile (Long et al., 2013b) stages, the larval phase seems the
1021 least affected by ocean acidification. It may be that the larvae are better adapted to changes in pH because
1022 of the environment they live in; many species of crustaceans have larvae that are tolerant of low pH
1023 (Kurihara and Ishimatsu, 2008; Arnold et al., 2009; McDonald et al., 2009; Pansch et al., 2012; Arnberg
1024 et al., 2013). The larval phase occurs in the water column and is timed to co-occur with the spring bloom
1025 when diel fluxuations in the water pH are likely to be relatively high. In addition, many crustacean larvae,
1026 including Tanner crab larvae (Wolotira et al., 1990), exhibit vertical migration, and there is a large
1027 difference in the carbonate chemistry between surface waters and those below the mixed layer in the
1028 Bering Sea during the period when Tanner Larvae are present (Cross et al., 2013). It is therefore likely
1029 that the planktonic larval phase of Tanner crabs is exposed to much higher variability in pH than are the
1030 other phases which occur only in the benthic environment, and is therefore better adapted to a greater
1031 range of pH levels than other stages may be. Similarly, Styf et al. (2013) argued that the insensitivity of
1032 *Nephrops norvegicus* embryos to low pH water was likely an adaptation to the naturally low pH
1033 environment in burrows in which the embryos are generally incubated in the wild.
1034
1035 Exposure to low pH during the embryo phase had a more substantial effect on Tanner crab larvae than
1036 exposure during the larval phase, and maternal exposure during the oogenesis phase increased the effect
1037 size. For larval morphometry, there were no differences among larval treatments for wild-brooded larvae;
1038 statistically, but not biologically, significant effects of embryo treatment in 2012; and both a statistically
1039 and biologically significant effect of embryo treatment in 2013. This matches results on the Tanner crab
1040 embryos (Swiney et al., in review); differences among pH treatments on embryo development were not
1041 biologically significant until the 2013 experiment. Similarly, the reduction in calcium and magnesium
1042 content and the increase in starvation survival time between the Ambient and pH 7.8 embryo treatments

were greater in 2013 than in 2012. Because of high mortality at the embryo and pre-zoea stages for the pH 7.5 embryo treatments (Swiney et al., in review), we can't explicitly compare these parameters between the years for the pH 7.5 embryo treatment; however, the higher mortality rate is a clear indication of substantial carryover effects despite the lack of data on non-lethal effect. This highlights the importance of carryover effects throughout the entire life history in the overall effects of ocean acidification, similar to those observed in other species (Hettinger et al., 2012; Schiffer et al., 2014). Many studies fail to account for carryover effects and thus likely underestimate the cumulative effects of ocean acidification (Hettinger et al., 2013).

The reduction in calcium and magnesium content and the Ca:Mg ratio could affect larval behavior and survival and is consistent with results at other life history stages; such a reduction also occurs in mature females (Swiney et al., in review), and juveniles (Long et al., 2013b), and occurs in larvae of other species, such as the European lobster, *Homarus gammarus* (Arnold et al., 2009). The lower calcium content in larvae likely decreases the strength of their exoskeleton. This could lead to increased vulnerability to predators (Amaral et al., 2012); a small change in prey vulnerability (Long et al., 2008) can lead to a large increase in predation (Long and Seitz, 2008) and a corresponding decrease in population size (Long et al., 2014). In addition, reduced hardness in the exoskeleton could lead to a decreased capacity to masticate food, which could also reduce larval survival. Although a lower Ca:Mg ratio is associated with stronger calcite (Magdans and Gies, 2004), any increase in hardness from this is unlikely to compensate for the large reduction in the overall content of both Ca and Mg.

Interestingly, the starvation survival time increased with decreasing pH. Larval starvation survival times are a function of the energetic reserves at hatching and the metabolic rate after hatching. At hatching, the total (not percent) carbon content of the larvae were similar among embryo treatments, indicating similar levels of lipid reserves, but the reduction over the 7 day incubation was greatest in ambient larvae. This suggests that larvae held under acidified conditions have a lower metabolic rate than those under ambient conditions, thus increasing their survival time. It is also noted that the significantly lower temperatures in the 2011 experiments may have affected this relationship but not in 2012 or 2013. Metabolic depression is a common response to ocean acidification among many taxa, likely because it reduces the hemolymph and intracellular acidity by reducing CO_2 production from respiration (Small et al., 2010; Christensen et al., 2011; Carter et al., 2013; Hennige et al., 2014). Although it does, as in this case, increase survival time under starvation conditions, it can have negative consequences for growth (Padilla-Gamino et al., 2013) and (in mature animals) reproduction.

1076
1077 This increase in starvation survival time contrasts with the response of larval red king crab, *Paralithodes*
1078 *camtschaticus*; they had a decreased starvation survival time indicating an increase in metabolic activity
1079 (Long et al., 2013a). There appears to be a dichotomy in the physiological response of crustaceans and
1080 other marine organisms to ocean acidification: Some crustaceans increase metabolism in order to
1081 maintain a constant internal pH, whereas other do not (Pane and Barry, 2007). Those that do may
1082 maintain pH homeostasis, as evidenced by the maintenance or even increase of calcification levels (Wood
1083 et al., 2008), but this results in lower growth and survival rates (Long et al., 2013a; Long et al., 2013b).
1084 Those that do not instead reduce metabolism and suffer internal hypercapnia, which can lead to reduced
1085 calcification, but have less of a reduction in growth and survival (Arnold et al., 2009; Walther et al., 2009;
1086 Walther et al., 2011). Tanner crabs appear to fall in the latter category.
1087
1088 These results, when combined with those on other life history stages, suggest that the effects of ocean
1089 acidification on the Tanner crab population and fishery are likely to be felt within 80-100 years. While the
1090 direct effects on larvae were relatively slight, effects on other life history stages, including the embryo,
1091 juvenile and adult stages, are larger (Long et al., 2013b; Swiney et al., in review). Further, the presence of
1092 carryover effects during the early life history stages suggest that it is likely carryover effects will continue
1093 to be felt in later stages and perhaps be magnified. Mortality at the juvenile, and to a lesser extent, the
1094 embryo stages is higher at pH 7.8, and even higher than that at pH 7.5, than at ambient pH (Swiney et al.,
1095 in review). A slightly higher mortality rate at the juvenile stage is predicted to cause a substantial decrease
1096 in the red king crab population and fishery before the end of the century (Punt et al., 2014) and we would
1097 expect a similar decrease for Tanner crabs commensurate with the slightly lower mortality rate.
1098
1099 A number of questions remain to be answered to fully understand the effects of ocean acidification on
1100 Tanner crabs. The gradual change in pH over decades will leave some potential for evolutionary
1101 adaptation. Even at the lowest pH tested, pH 7.5, some larvae, embryos, and juveniles were able to
1102 survive and grow, indicating a range in the individual tolerances for low pH (Long et al., 2013a; Long et
1103 al., 2013b; Swiney et al., in review) and the potential for natural selection to increase the average fitness
1104 of the population under acidified conditions over time (Reusch, 2014). Indirect effects, such as changes in
1105 predation on Tanner crabs or in their ability to feed, may significantly alter the net effect of ocean
1106 acidification on this species. In addition, other stressors, such as increasing temperatures, may interact
1107 with ocean acidification. Further research will be necessary to inform policymakers of the likely effects

1108 that ocean acidification will have on this important commercial species and the best management steps to
1109 protect this resource.
1110
1111 **Acknowledgements**
1112 This project was funded by the North Pacific Research Boart (NPRB publication no. XXX) and National
1113 Marine Fisheries Service (NMFS). We thank NMFS biologists and laboratory technicians for their
1114 assistance which made this project possible. Previous versions of this paper were improved by comments
1115 from XXXXXXXX. The findings and conclusions in the paper are those of the authors and do not
1116 necessarily represent the views of the National Marine Fisheries Service, NOAA. Reference to trade
1117 names does not imply endorsement by the National Marine Fisheries Service, NOAA.
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1119

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Table 2.1: Average water chemistry parameters in experimental units (± 1 standard deviation) during experiments on larval Tanner crabs for each of the three years. Temperature and pH were measured daily, DIC (all years) and alkalinity (2012 and 2013 only) were measured weekly, and all other parameters were calculated.

Treatment	Temperature °C	pH	pCO_2 µatm	HCO_3^- mmol/kg	CO_3^{-2} mmol/kg	DIC mmol/kg	Alkalinity mmol/kg	$\Omega_{Aragonite}$	$\Omega_{Calcite}$
2011									
Ambient	4.66 ± 0.19	8.21 ± 0.04	269.42 ± 20.45	1.81 ± 0.01	0.12 ± 0.01	1.95 ± 0.00	2.13 ± 0.01	1.88 ± 0.09	3.00 ± 0.14
pH 7.8	4.61 ± 0.13	7.79 ± 0.02	810.33 ± 22.89	1.97 ± 0.00	0.05 ± 0.00	2.06 ± 0.00	2.10 ± 0.01	0.76 ± 0.03	1.21 ± 0.05
pH 7.5	4.63 ± 0.17	7.49 ± 0.02	1665.48 ± 161.70	2.02 ± 0.02	0.03 ± 0.00	2.14 ± 0.01	2.09 ± 0.02	0.39 ± 0.03	0.62 ± 0.05
2012									
Ambient	6.85 ± 0.66	8.16 ± 0.04	345.66 ± 34.66	1.87 ± 0.01	0.11 ± 0.01	2.00 ± 0.01	2.16 ± 0.06	1.70 ± 0.13	2.71 ± 0.21
pH 7.8	6.58 ± 0.43	7.81 ± 0.02	787.30 ± 21.50	2.02 ± 0.02	0.06 ± 0.00	2.11 ± 0.02	2.17 ± 0.05	0.85 ± 0.03	1.36 ± 0.04
pH 7.5	6.69 ± 0.50	7.50 ± 0.02	1643.71 ± 91.51	2.03 ± 0.04	0.03 ± 0.00	2.14 ± 0.05	2.23 ± 0.14	0.42 ± 0.02	0.66 ± 0.04
2013									
Ambient	7.01 ± 0.55	8.16 ± 0.03	326.53 ± 33.75	1.85 ± 0.01	0.12 ± 0.01	1.98 ± 0.01	2.12 ± 0.01	1.76 ± 0.16	2.81 ± 0.25
pH 7.8	7.12 ± 0.59	7.79 ± 0.01	811.36 ± 38.31	1.99 ± 0.02	0.05 ± 0.00	2.09 ± 0.02	2.09 ± 0.02	0.81 ± 0.04	1.29 ± 0.07
pH 7.5	7.06 ± 0.54	7.50 ± 0.02	1619.80 ± 59.85	2.05 ± 0.02	0.03 ± 0.00	2.15 ± 0.02	2.13 ± 0.01	0.43 ± 0.02	0.69 ± 0.03

Table 2.2: Ranking of models of Tanner crab larvae mortality in 2011 using AIC_c and the parameter estimates ± standard error for the best fit models. Model indicates the parameters used and how they were modeled (see text for details). Where factors are included parenthetically, the parameter is modeled as a linear function of those parameters. T indicates that the parameter varies among all treatments and T2 indicates the post-hoc models in which the parameter was the same for the ambient (A) and pH 7.8 treatments which differed from the pH 7.5 treatment. K is the number of parameters for each model. To show the greatest difference among the treatments supported by the data, the more complex t_{50}(T2)s(T) model is presented in figure 2.2. The t_{50} parameters have units of days and the s parameters are unitless.

Model	**K**	**AIC_c**	**ΔAIC_c**	**Likelihood**	**AIC_c Weight**
t_{50}, s	2	923.39	4.01	0.13	0.05
t_{50}(T), s	4	924.39	5.01	0.08	0.03
t_{50}(T2), s	3	922.85	3.47	0.18	0.07
t_{50}, s(T)	4	919.38	0.00	1.00	0.40
t_{50}(T)s(T)	6	921.79	2.41	0.30	0.12
t_{50}(T2)s(T)	5	919.84	0.46	0.80	0.32

	Parameter Estimates	
Parameter	**t_{50}, s(T)**	**t_{50}(T),s(T2)**
t_{50}	9.47 ± 0.07	-
t_{50}(A, pH 7.8)	-	9.54 ± 0.09
t_{50} (pH 7.5)	-	9.36 ± 0.11
s(A)	-6.18 ± 0.32	-6.22 ± 0.33
s(pH 7.8)	7.12 ± 0.37	-7.16 ± 0.38
s(pH 7.5)	7.6 ± 0.41	-7.53 ± 0.41

Table 2.3: Ranking of models of Tanner crab larvae mortality in 2012 and 2013 using AIC_c. Model indicates the parameters used and how they were modeled (see text for details). Where factors are included parenthetically, the parameter is modeled as a linear function of those parameters. E indicates that the parameter varies as a function of the treatment during the embryo stage, L indicates that the parameter varies as a function of the treatment during the larval stage, # indicates that the two factors were fully crossed, * indicates a post hoc model in 2012 where select parameters from the fully crossed model were removed because the estimates were close to 0. K is the number of parameters for each model.

		2012				**2013**			
Model	**K**	**AIC_c**	**ΔAIC_c**	**Likelihood**	**AIC_c Weight**	**AIC_c**	**ΔAIC_c**	**Likelihood**	**AIC_c Weight**
t_{50}, s	2	6026.22	605.59	0.00	0.00	5632.03	816.42	0.00	0.00
t_{50}(E), s	4	5552.36	131.74	0.00	0.00	5256.96	441.35	0.00	0.00
t_{50}(L), s	4	6027.39	606.76	0.00	0.00	5529.52	713.91	0.00	0.00
t_{50}(E,L), s	6	5553.26	132.64	0.00	0.00	5139.66	324.05	0.00	0.00
t_{50}(E#L), s	10	5513.51	92.89	0.00	0.00	5081.42	265.82	0.00	0.00
t_{50}, s(E)	4	6020.42	599.79	0.00	0.00	5612.98	797.37	0.00	0.00
t_{50}, s(M)	4	6027.36	606.73	0.00	0.00	5624.00	808.39	0.00	0.00
t_{50}, s(E,M)	6	6021.50	600.88	0.00	0.00	5605.75	790.15	0.00	0.00
t_{50}, s(E#M)	10	6019.86	599.24	0.00	0.00	5531.20	715.59	0.00	0.00
t_{50}(E#L), s(E#M)	18	5430.33	9.71	0.01	0.01	4815.61	0.00	1.00	1.00
t_{50}(E#L), s(E#M)*	12	5420.63	0.00	1.00	0.99	-	-	-	-

Table 2.4: Parameter estimates for logistic regressions on larval survival experiments in 2012 and 2013 graphed in Fig. 2.5. Embryo and Larvae indicates the treatments at the embryo and larval stages and include A- Ambient, 7.8- pH 7.8, and 7.5- pH 7.5. The t_{50} parameters have units of days and the s parameters are unitless.

Embryo	A	A	A	7.8	7.8	7.8	7.5	7.5	7.5
Larvae	A	7.8	7.5	A	7.8	7.5	A	7.8	7.5
	2012								
t_{50}	9.52	9.09	9.80	9.24	8.81	9.52	12.80	13.14	11.50
s	-3.13	-3.13	-3.13	-2.86	-2.86	-3.18	-3.93	-3.93	-3.54
	2013								
t_{50}	12.42	11.36	10.44	14.54	14.05	12.06	15.58	13.28	15.31
s	-3.81	-4.01	-3.44	-4.86	-5.31	-3.39	-5.10	-4.25	-7.25

Table 2.5: Results of two-way fully crossed ANOVAs on carbon (C), nitrogen (N), Calcium (Ca), and magnesium (Mg) content, the C:N and Ca:Mg ratios, and larval mass in Tanner crab larvae. Treatments were at the embryo (E) and larval (L) stages.

	2012						**2013**					
	Embryo		**Larval**		**E*L**		**Embryo**		**Larval**		**E*L**	
	F	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**	**F**	**p**
C	9.03	< 0.0005	104.11	< 0.0005	1.50	0.20	19.35	< 0.0005	54.73	< 0.0005	1.21	0.32
N	18.09	< 0.0005	165.23	< 0.0005	2.30	0.05	52.77	< 0.0005	84.93	< 0.0005	1.45	0.25
C:N	3.17	0.05	2.39	0.08	0.79	0.59	9.90	< 0.0005	5.45	0.00	1.16	0.34
Ca	9.36	< 0.0005	3.74	0.02	0.72	0.63	168.25	< 0.0005	63.79	< 0.0005	1.95	0.14
Mg	8.62	0.00	105.35	< 0.0005	1.34	0.26	2.04	0.16	12.77	< 0.0005	0.28	0.84
Ca:Mg	3.19	0.05	7.86	< 0.0005	0.56	0.76	405.70	< 0.0005	130.41	< 0.0005	13.17	< 0.0005
Mass	0.36	0.70	19.09	< 0.0005	0.42	0.86	6.62	0.02	19.80	< 0.0005	0.49	0.69

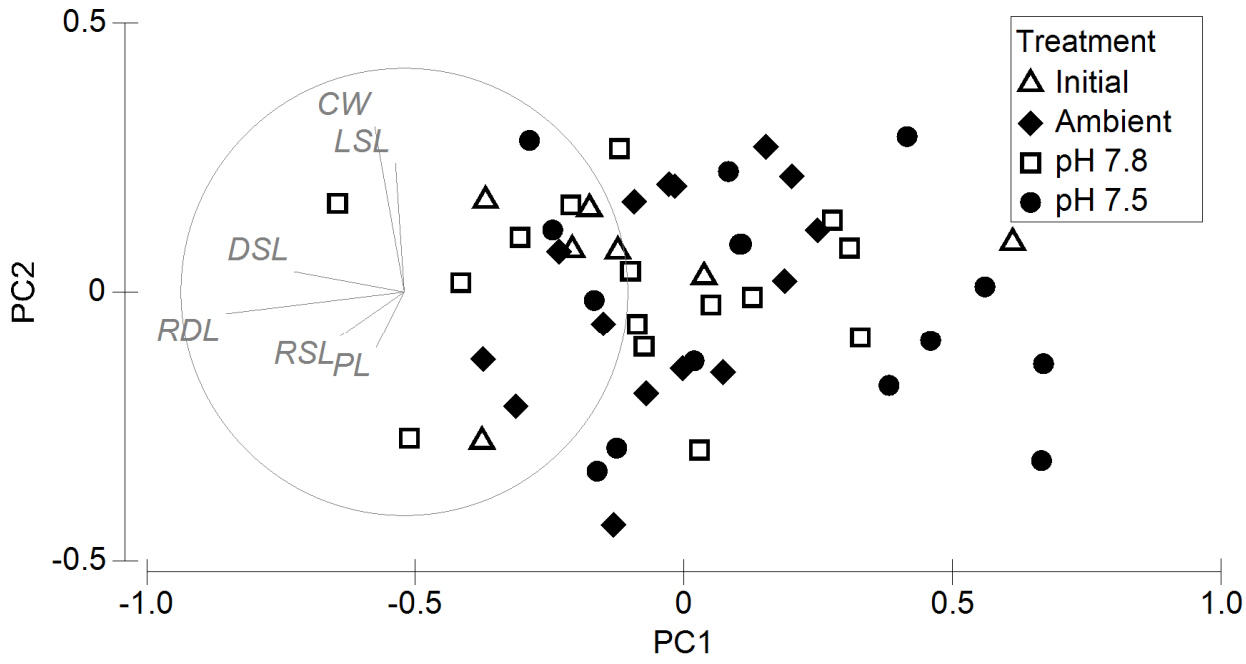

Figure 2.1: Principle component analysis of 2011 Tanner crab larvae morphometrics. Initial represents larvae measured the day after hatching. Ambient, pH 7.8, and pH 7.5 represent larvae that had been held in control and acidified water for 10 days. The vector plot shows the loadings for the 6 different measurements made on each larva: CW- carapace width, LSL- lateral spine length, DSL- dorsal spine length, RDL- rostro-dorsal length, RSL- rostral spine length, and PL- protopodite length. PC 1 explains 51.8% of the data and PC 2 explains 26.8% of the data.

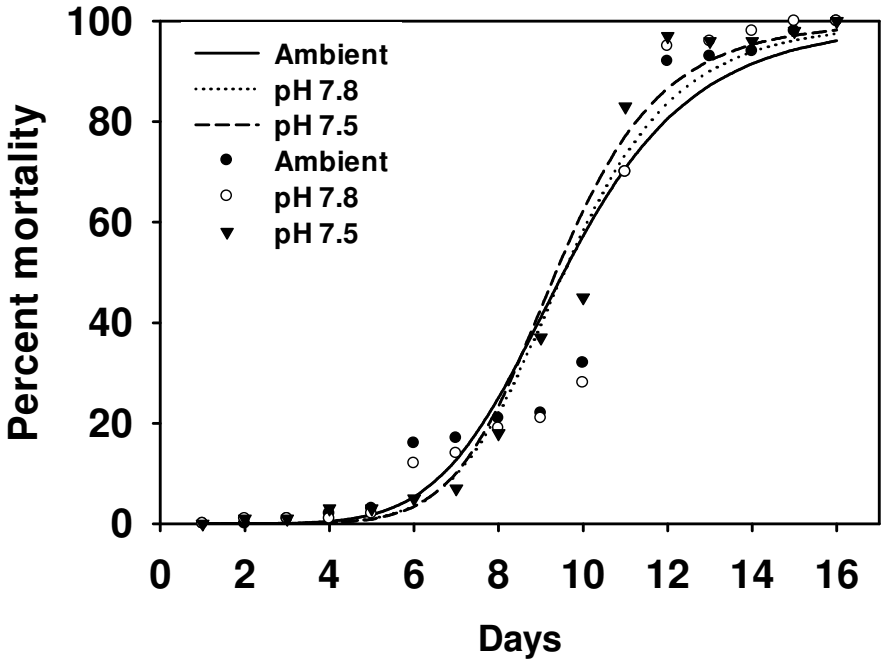

Figure 2.2: Mortality of Tanner crab larvae in 2011 at three pH treatments over time. Points are the average percent mortality at each treatment and lines are the best fit logistic regression model for each (see Table 1 for parameter estimates).

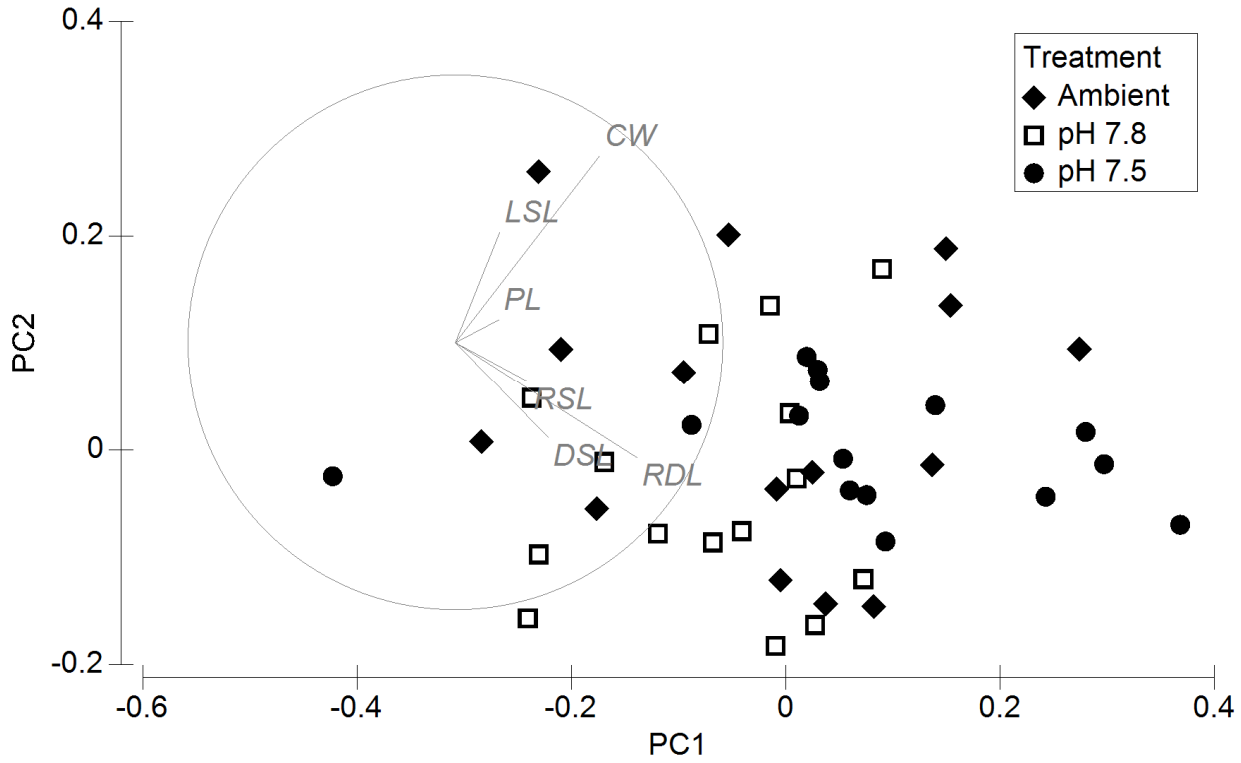

Figure 2.3: Principle component analysis of newly hatched 2012 larvae morphometrics. The vector plot shows the loadings for the 6 different measurements made on each larva: CW- carapace width, LSL- lateral spine length, DSL- dorsal spine length, RDL- rostro-dorsal length, RSL- rostral spine length, and PL- protopodite length. PC 1 explains 56.8% of the data and PC 2 explains 22.7% of the data.

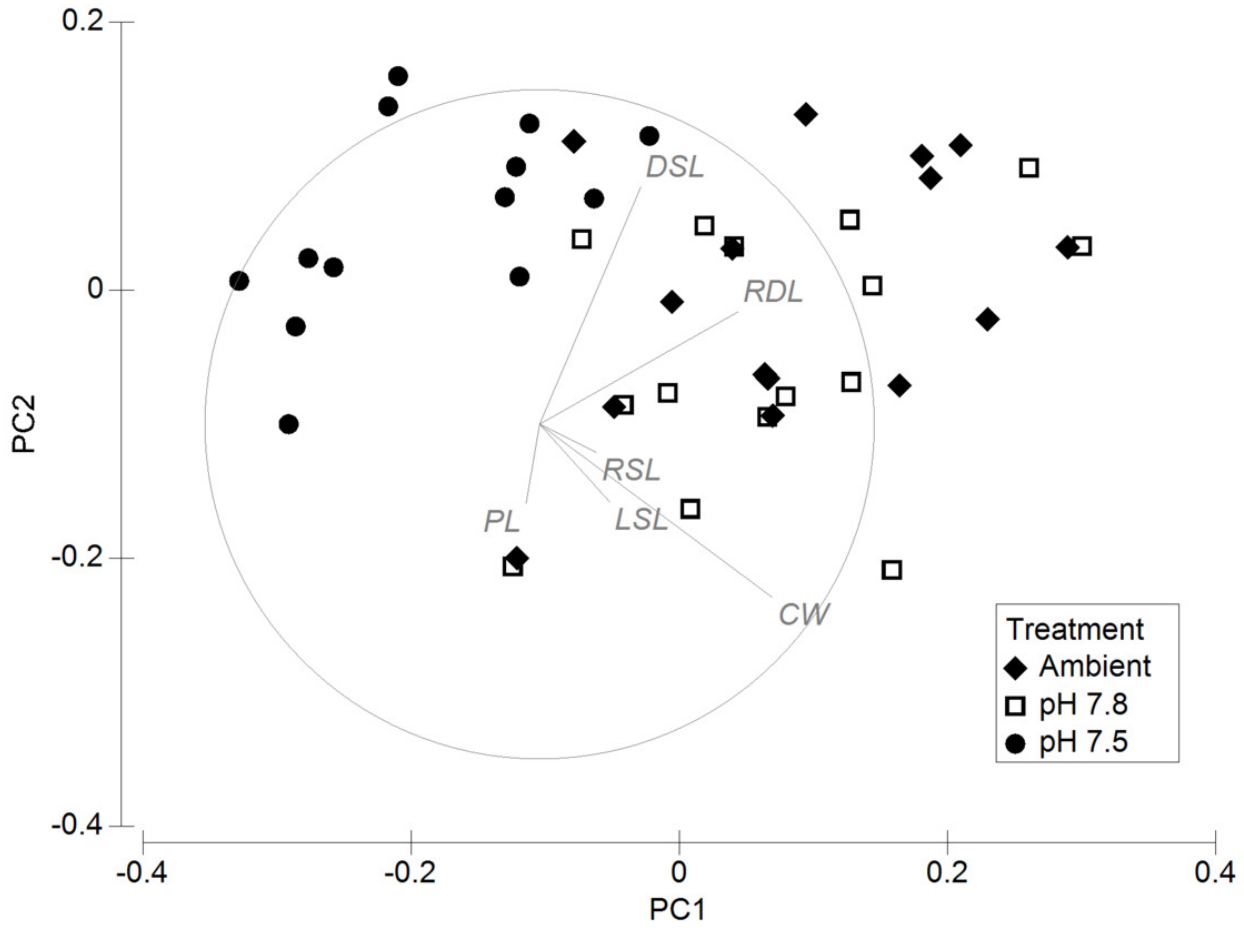

Figure 2.4: Principle component analysis of newly hatched 2013 larvae morphometrics. The vector plot shows the loadings for the 6 different measurements made on each larva: CW- carapace width, LSL- lateral spine length, DSL- dorsal spine length, RDL- rostro-dorsal length, RSL- rostral spine length, and PL- protopodite length. PC 1 explains 56.2% of the data and PC 2 explains 19.1% of the data.

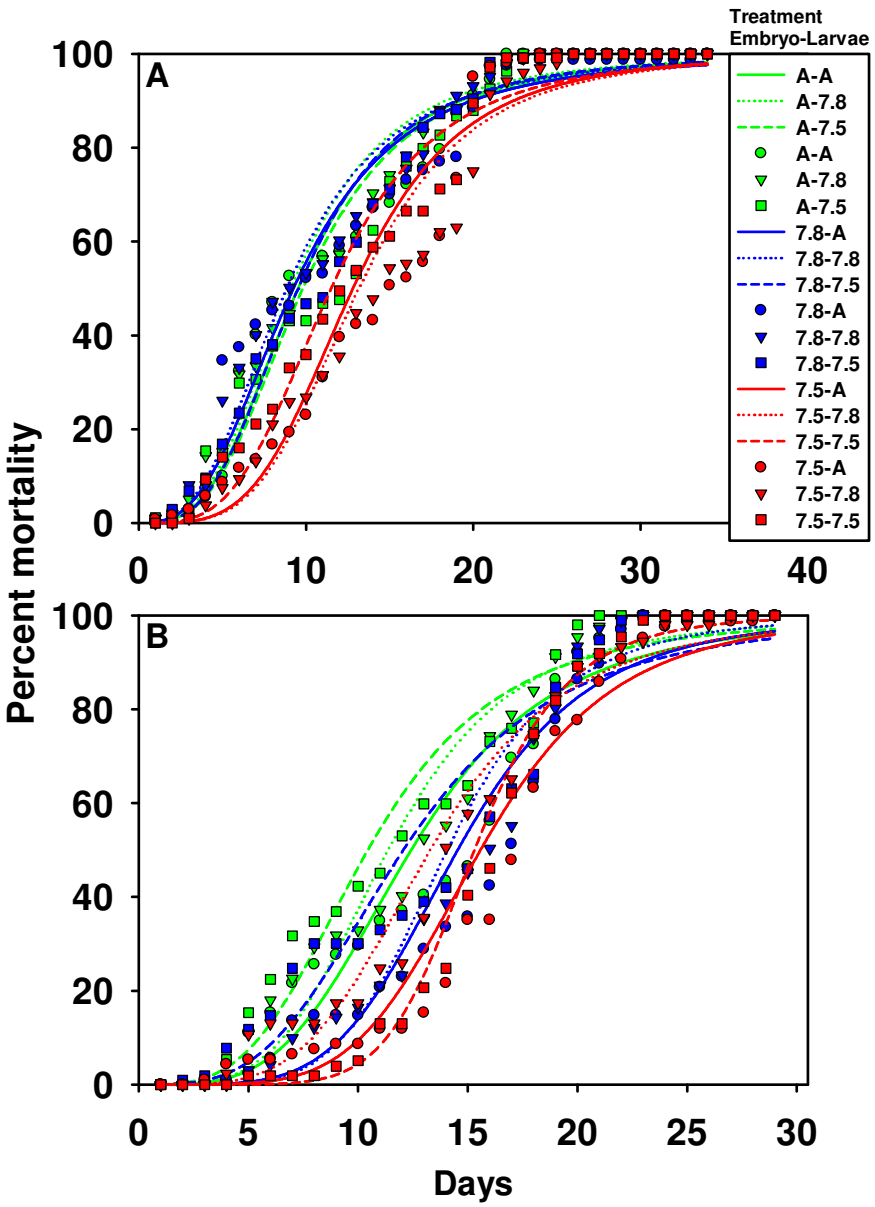

Figure 2.5: Mortality of Tanner crab larvae in survival experiments at three pH treatments over time in A) 2012 and B) 2013. Points are the average percent mortality at each treatment and lines are the best fit logistic regression model for each (see Table 3 for parameter estimates). The treatments are A- Ambient, 7.8- pH 7.8, and 7.5- pH 7.5. The treatment represents the treatment the larvae were in during embryonic development and the second the treatment they were in as larvae.

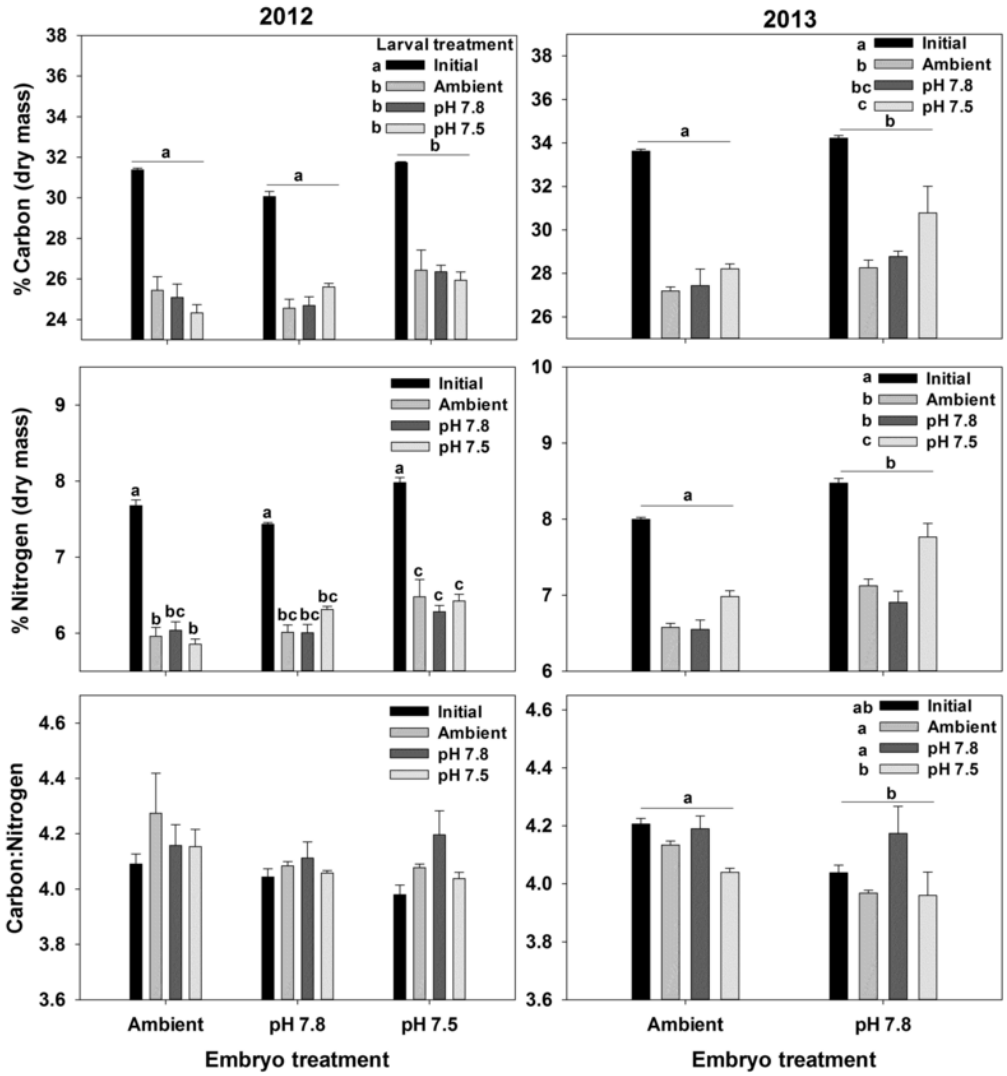

Figure 2.6: Effects of exposure to acidified water at the embryo and larval stages on carbon and nitrogen content and the C:N ratio in Tanner crab larvae. Bars are the mean + 1 standard error. For the larval treatments, Initial represents the larvae immediately after hatching and Ambient, pH 7.8, and pH 7.5 represent the treatment larvae were held in for 7 days. Bars with different letters above them differ significantly. Differences among the larval treatments are indicated with letters next to the legend. Results for the 2012 experiments are in the left side plots and those for the 2013 experiments are on the right side plots.

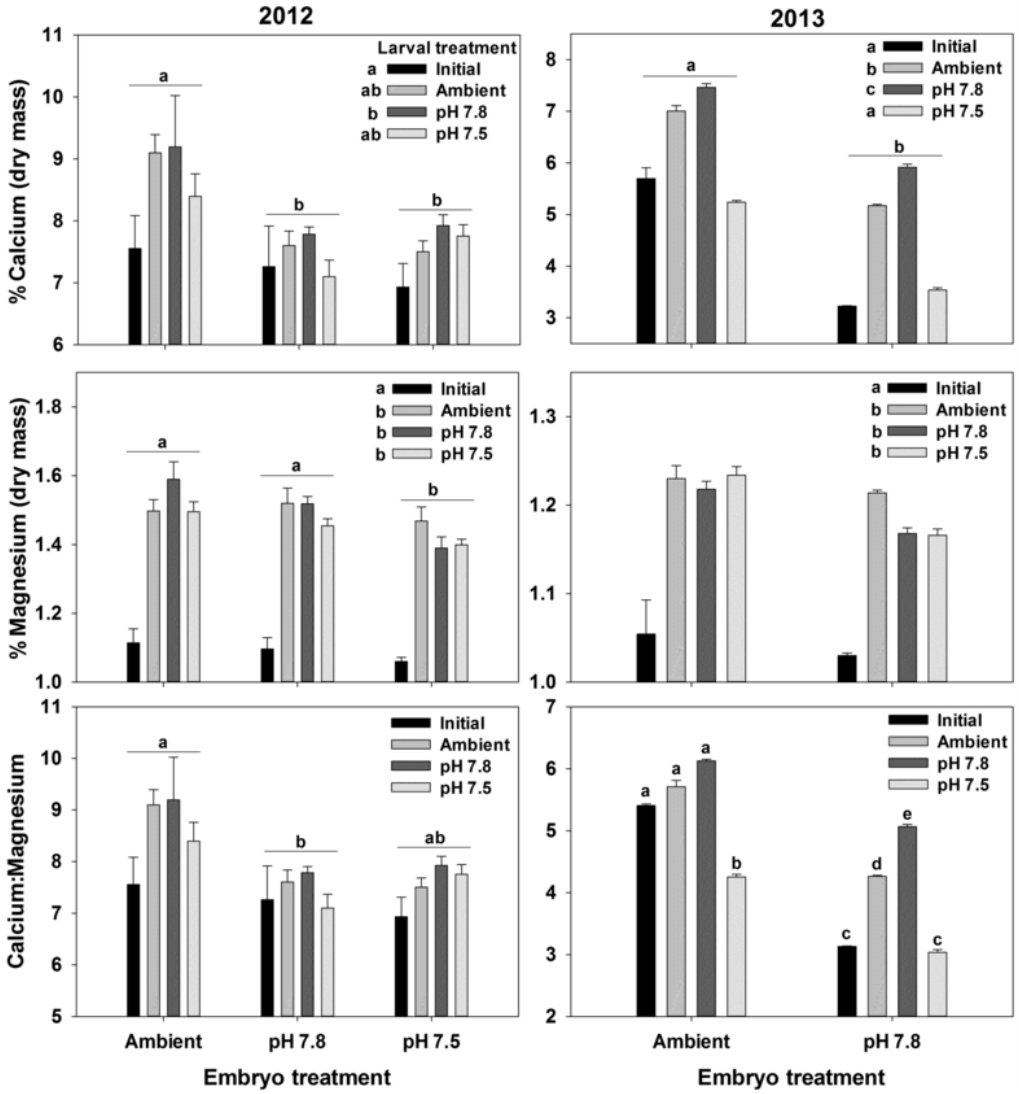

Figure 2.7: Effects of exposure to acidified water at the embryo and larval stages on calcium and magnesium content and the Ca:Mg ratio in Tanner crab larvae. Bars are the mean + 1 standard error. For the larval treatments, Initial represents the larvae immediately after hatching and Ambient, pH 7.8, and pH 7.5 represent the treatment larvae were held in for 7 days. Bars with different letters above them differ significantly. Differences among the larval treatments are indicated with letters next to the legend. Results for the 2012 experiments are in the left side plots and those for the 2013 experiments are on the right side plots.

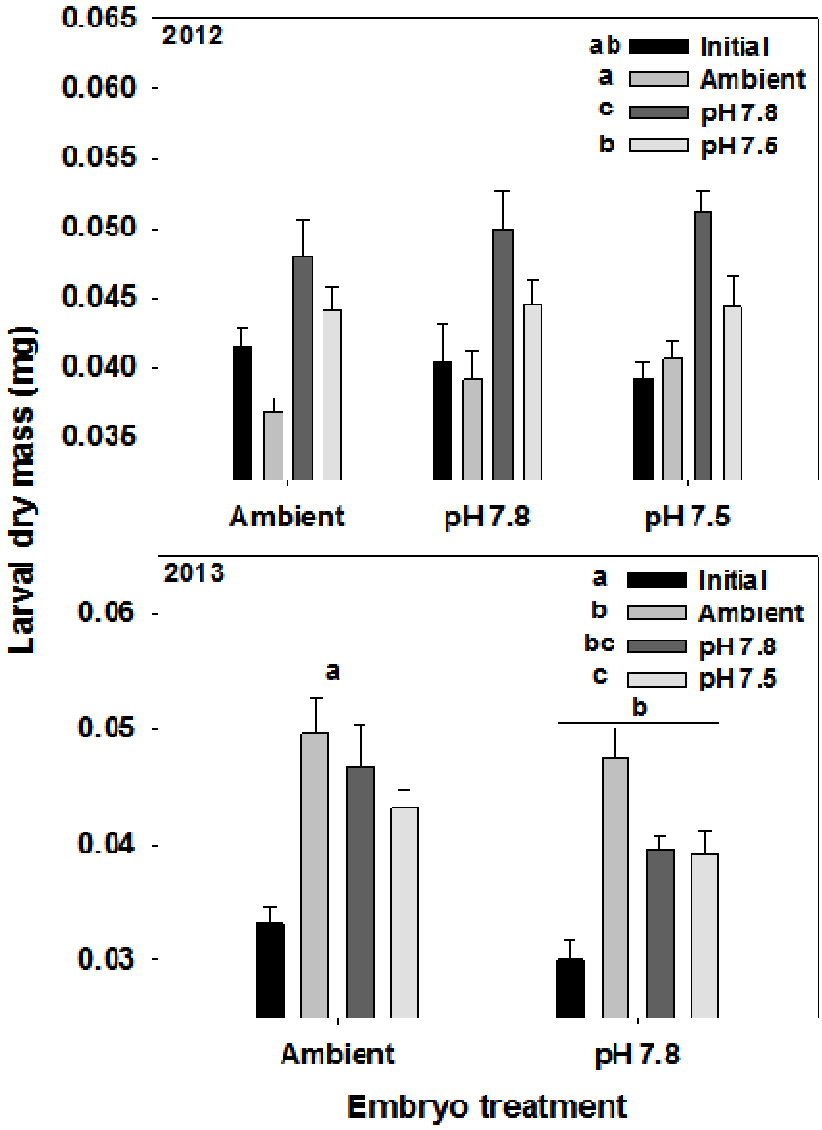

Figure 2.8: Effects of exposure to acidified water at the embryo and larval stages on the mass of Tanner crab larvae in 2012 and 2013 experiment. Bars are the mean + 1 standard error. For the larval treatments, Initial represents the larvae immediately after hatching and Ambient, pH 7.8, and pH 7.5 represent the treatment larvae were held in for 7 days. Bars with different letters above them differ significantly. Differences among the larval treatments are indicated with letters next to the legend.

Conclusions

Laboratory experiments on early life stages of Tanner crab exposed to ocean acidification (decreased pH and increased pCO_2) show that without local acclimation, crab populations may be negatively affected by predicted ocean acidification conditions. In crab only exposed to acidification after embryo extrusion, both embryological and larval effects were minimal. However, when oocyte development was included in the period of exposure, significant effects in embryo and larval development were found. Long term exposure of ocean acidification conditions led to negative effects on development, the number of viable larvae hatched, and overall hatching success in the embryological stage. Hatch duration showed no effects. At the larval stage, morphometry was significantly different among treatments and crabs were smaller at lower pH. Larval survival duration during starvation experiments was longer in the lower pH treatment suggesting a reduced metabolism response mechanism. Calcium content was decreased with ocean acidification conditions in both the embryo and larval stages. The consistency in this pattern with the juvenile stage (Long et al., 2013b) may be due to a response to the duration of this experiment or to physiological processes in Tanner crab. In all, the responses found in the second year of the study versus the first highlight the importance of cumulative effects when determining the physiological response of organisms to ocean acidification. Additional considerations for other environmental conditions such as temperature will also need to be studied as a cumulative effect.

As with other research (Kurihara, 2008), this study also identifies the need to assess multiple life history stages to understand the full potential for population level effects of ocean acidification. In the case of Tanner crab, the larval stage (Manuscript #2; page 34) was least affected by ocean acidification when compared to the embryo (Manuscript #1; page 9) or juvenile (Long et al., 2013b) stages. The resilience of the larval stage may suggest an adaptive strategy already incorporated into this life history stage. Therefore, spatial variability in the exposure of different life history stages will be critical to understanding the ultimate effects that ocean acidification will have on crab stocks in Alaska.

Management or policy implications

The results of these physiological response experiments suggest that Tanner crab populations may be negatively affected by ocean acidification. However, the variable response of different life stages and the

unknown environmental variability in ocean acidifications at scales important to Tanner crab leave room for speculation as to how Tanner crab populations will respond to the increases in ocean acidification expected in the North Pacific. In addition to the unknowns identified above, the unknown adaptive and ecosystem responses of Tanner crab to ocean acidification suggest that further research is necessary to understand the scale of response expected from different Tanner crab life stages. Lastly, *in situ* data collection is required to inform physiological response experiments and population models so that policymakers can consider the likely effects that ocean acidification will have on this important commercial species and the best management steps to protect this resource.

Publications:

Swiney, K.M., W.C. Long, R.J. Foy. In prep. Ocean acidification alters embryo development and reduces the number of viable larvae hatched, and calcification in Tanner crab, *Chionoecetes bairdi*.

Long, W.C., K.M. Swiney, R.J. Foy. In prep. Effects of ocean acidification on Tanner crab larvae: Carryover effects from oogenesis and embryogenesis are stronger than direct effects.

Outreach

Outreach for this study consisted of a number of scientific and public presentations. In addition, numerous fixed displays at the Kodiak Fisheries Research Center were developed to educate the public about ocean acidification, the response of crab stocks in Alaska, and the experimental system developed at the Kodiak Laboratory; all supported by the North Pacific Research Board. The Kodiak Fisheries Research Center (KFRC) has over 12,000 visitors annually from both the Kodiak community and visitors from throughout the world.

R.J. Foy, W.C.Long, and K.M. Swiney. The effects of ocean acidification on maternal condition and reproductive success and larval condition and survival of Tanner crabs, Chionoecetes bairdi. Alaska Marine Science Symposium. Anchorage, AK. January 16-20, 2012.

W.C.Long, K.M. Swiney, and R.J. Foy. Effects of ocean acidification on red king crab and Tanner crab. National Shellfisheries Annual Meeting. Seattle, WA. March 25-29, 2012.

R.J. Foy, W.C.Long, and K.M. Swiney. The effects of ocean acidification on maternal condition and reproductive success and larval condition and survival of Tanner crabs, Chionoecetes bairdi. COMFISH. Kodiak, AK. April 12-14, 2012. This is Alaska's largest fisheries trade show and a public forum presentation.

R.J. Foy. Science across the bridge: NMFS research at the Kodiak Fisheries Research Center. COMFISH. Kodiak, AK. April 12-14, 2012. This is a public forum presentation and NPRB funded ocean acidification research was highlighted.

R.J. Foy. Regional fisheries environmental science and water quality. Kodiak Archipelago Rural Regional Leadership Forum. May 3, 2012. This is a group representing native communities in Alaska and concerned about climate change.

R.J. Foy, M. Carls, M. Dalton, T. Hurst, W. C. Long, D. Poljak, A. E. Punt, M. F. Sigler, R. P. Stone, K. M. Swiney. 2013. CO2, pH, and anticipating a future under ocean acidification. Onchrhynchus XXXIII: 1-5.

Seattle Times. A Sea Change. September 2013. This was a multi-page, multi-edition article by Craig Welch highlighting ocean acidification research in the west coast and Alaska. In particular the NPRB and NOAA funded king and Tanner crab research at the Kodiak Laboratory was described.

W.C.Long, K.M. Swiney, and R.J. Foy. Effects of Ocean Acidification on Tanner Crab, Chionoecetes bairdi, Larvae. Alaska Marine Science Symposium. Anchorage, AK. January 19-23, 2014.

Long, W.C., K.M. Swiney, R.J. Foy, 2014. Effects of ocean acidification on embryo development and fecundity in Tanner crab, *Chionoecetes bairdi*. Alaska Marine Science Symposium. Anchorage, Alaska, January 19-23, 2014.

R.J. Foy. Bering Sea Crab Resources: Management, Assessment, and Science. Aleutian, Pribilof, Island Community Development Association-Community Outreach Conference. April 3, 2014. This is a group representing native communities in Alaska and concerned about ocean acidification and fisheries.

Acknowledgements

We thank the North Pacific Research Board for funding this project and we thank the NOAA Ocean Acidification Program for continued support to develop and maintain infrastructure at the Kodiak

Laboratory to conduct experiments on ocean acidification. We thank the NOAA biologists and laboratory technicians for their assistance in collecting and caring for experimental crabs. In particular, we thank Matt Dawson, Ashley Emley, Nikki Gabriel, Lacey Harver, Alyssa Hopkins, Jane Murawski, and Nick Sisson for laboratory assistant; Pete Cummiskey and Eric Munk for crab collections; and Mark Carls and Lawrence Schaufler for assistance with analytical chemistry. Additional seawater chemistry was conducted by Jeremy Mathis at the University of Alaska Ocean Acidification lab.

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