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Paths to growth: Exploring the effects of reduced pH and increased temperature on a fisheries-important prawn

A Thesis submitted in partial satisfaction of the requirements
for the degree Master of Science

in

Marine Biology

by

Zoe Camille Sebright

Committee in charge:

Jennifer R. A. Taylor, Chair
Dimitri Deheyn
Martin Tresguerres

2019

The Thesis of Zoe Camille Sebright is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

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Chair

University of California San Diego

2019

DEDICATION

I am dedicating my work in this thesis to my family, both chosen and not. First to my father, Scott Sebright who is the only reason for my ability to attain an education he was not given the opportunity to possess. I hope to repay the favor or pay it forward in time. To my mother, Bonnie Sebright, for her strong genes and unwavering support that got me through the past five years. To my sisters Chloe Larriategui, Sophie Sebright, and Lily Sebright for their love and for being the ultimate motivation for the accomplishment of my goals. Finally, to my chosen family member, Sarah Mcknight, one of the only people I trust enough to ask for and accept help from at every turn, including throughout this entire process.

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ABSTRACT OF THE THESIS

Paths to growth: Exploring the effects of reduced pH and increased temperature on a fisheries-important prawn

by

Zoe Camille Sebright

Master of Science in Marine Biology

University of California San Diego, 2019

Professor Jennifer Taylor, Chair

Crustaceans are relatively understudied in regards to their vulnerability to the changing ocean conditions of ocean acidification and ocean warming. Although they are generally considered less vulnerable to reduced pH and increased temperature than other calcifying groups, studies have found potential effects on their growth, energy storage, and prey detection. In this study, we examined the vulnerability of the ridgeback prawn, *Sicyonia ingentis*, which is a commercially important species along the West coast of the United States. Prawn were exposed to reduced pH (7.50 ± 0.02; pCO_2 = 1475 ± 25

μatm) and increased temperature (16.2 ± 0.7°C) conditions in a full factorial design for twelve weeks. Prawns were monitored for survival and growth throughout the experiment. At the end of the experiment, their prey detection was analyzed via antennular flicking rates, and they were dissected for Gonadosomatic Index (GSI) and Hepatosomatic Index (HSI) measurements, which are indicators of gonad development, energy storage, and the trade-off between the two. No significant effect of treatment was found for antennular flicking, GSI, or HSI. The second molt increment was significantly less in the reduced pH/increased temperature treatment in comparison to the control (ANOVA: $F_{3,18} = 3.36$, $p = 0.04$), but growth over the experiment did not differ among treatments. Survival was significantly lower in the reduced pH/increased temperature treatment. *S. ingentis* is robust to a pH below its natural range, but the synergistic effects of reduced pH and increased temperature have a significant impact on mortality.

INTRODUCTION

Climate change is creating new challenges for marine life, particularly in regard to ocean acidification and ocean warming. Ocean acidification is the change in seawater chemistry resulting from absorption of atmospheric CO_2. This increase in ocean pCO_2 enables the dissociation of bicarbonate ions to produce H+ ions, reducing seawater pH, carbonate ion concentration, and calcium carbonate saturation states. Continued carbon uptake by the ocean is virtually certain to exacerbate ocean acidification, with projections ranging from a 0.2-0.3 unit decrease in open ocean surface pH by the end of this century (IPCC, 2014).

As the threat of ocean acidification continues, marine organisms have displayed large variation in their responses to ocean acidification-like conditions (Kroeker et al., 2010). Calcifying organisms are broadly seen as a group most impacted by reduced pH conditions, although this is variable and depends on the nature of the calcification process, the type of calcium carbonate used in calcification, the exposure of the calcifying space to seawater, and the amount of calcium carbonate in the outer shell or exoskeleton (Kroeker et al., 2011; Whiteley, 2011). The impacts of ocean acidification are hypothesized to be more severe in sessile species, such as corals and mollusks, more so than mobile species, such as crustaceans and fish. This is due to the increased metabolism of species that are more mobile (Portner, 2008).

Co-occurring with changing seawater chemistry is ocean warming. Increasing levels of atmospheric CO_2 are increasing the average global sea surface temperature,

which is predicted to increase by 2-3 degrees before the end of the century (IPCC, 2014). Elevated temperatures can increase the metabolic rate of organisms within their thermal tolerance window and can enable the rapid deterioration of cellular processes and performance beyond this thermal limit (Portner, 2008). This poses a challenge in predicting the potential effects on marine organisms of ocean warming in concurrence with ocean acidification. Thus, research exploring the combined effects of ocean acidification and warming has been on the rise. It is possible that ocean warming offsets the effects of ocean acidification, such as mitigating the negative effects of reduced pH on calcification (McCulloch et al., 2012). Yet, any offsets created by increasing temperature would be limited to the organism's thermal tolerance (Portner, 2008). There is also the potential for the additional stress effect of ocean warming to aggravate the effects of ocean acidification (Anthony et al., 2008). A meta-analysis found a trend towards lower survival, growth, and development under elevated temperatures in combination with reduced pH, but no mitigating or synergistic effects of elevated temperature on reductions in calcification caused by reduced pH (Kroeker et al., 2013).

To date, ocean acidification and ocean warming research has underemphasized crustaceans, in part because they are hypothesized to be robust to changing ocean carbon chemistry. Crustaceans have been shown to be physiologically more capable of tolerating the reduced pH conditions associated with ocean acidification (Whiteley, 2011). This robustness against changes in environmental pH is attributed to their use of bicarbonate ions and metabolic CO_2 in the calcification process (Ries et al., 2009), an effective acid-

base regulatory system to buffer changes to hemolymph pH (Roleda et al., 2012), and their use of the less soluble form of calcite (BoBelmann et al., 2007).

Despite having these physiological advantages, experimentally reduced pH conditions can cause a variety of effects on a diversity of crustacean species. For example, under reduced pH conditions, crustaceans have been shown to experience negative growth rates (Kurihara, 2008; Wickens, 1984), decreased intermolt duration (Wickens, 1984), increased molt frequency (Small et al., 2016), increased calcification (Wickens, 1984), decreased calcification (Long et al., 2013), reduced prey detection (de La Haye et al., 2011; Kim et al., 2015), and reduced antennular flicking (de La Haye et al., 2011; Kim et al., 2015; Tierney & Aetma, 1985). Yet, unlike echinoderms, cnidarians, and mollusks, crustaceans do not demonstrate consistent effects when exposed to ocean acidification and ocean warming conditions. For example, in contrast to the studies referenced above, other crustacean species have shown no effect on growth (deVries et al., 2016; Taylor et al., 2015), no effect on molting (deVries et al., 2016; Lowder et al., 2017; Taylor et al., 2015), and no effect on calcification (Lowder et al., 2017) under experimentally reduced pH.

Early life history stages are considered to be more vulnerable to ocean acidification and warming conditions (Kurihara et al., 2008), however, the effects on growth in particular are commonly observed in both early and mature stages of crustaceans. For example, juveniles (small, post-larvae) of two species of prawn, *Penaeus occidentalis* and *P. monodon,* both experienced decreased growth under exposure to reduced pH for 56 and 36 days, respectively, although the effects were only seen at pH

levels below 7.74 (Wickens, 1984). *P. occidentalis* experienced a decrease in average weight gain of 0.4 grams, while *P. monodon* experienced a decrease of 0.96 grams (Wickens, 1984). Yet, adult shrimp *Palaemon pacificus* experienced a reduction in total length of 0.2 mm, on average, after 6 weeks of exposure to a reduced pH of 7.64 (Kurihara et al., 2008). These significant effects on growth at different life stages can affect the survival and reproductive potential of crustaceans and is therefore of considerable concern for future populations. In contrast, mature individuals of other species, such as the shrimp *Lysmata californica* and the mantis shrimp *Neogonodactylus bredini*, did not exhibit changes in growth under the reduced pH conditions predicted for the end of the century (deVries et al., 2016; Taylor et al., 2015).

Variation among species within taxonomic groups has generally been attributed to differences in life history and geographic distribution. Crustaceans have been shown to differ in responses to reduced pH based on life stage (Kroeker et al., 2010). Also, experimental pH, methods of reducing pH, and experiment duration vary across studies, which makes it difficult to compare results and establish general patterns. It is possible that the variance in reactions to ocean acidification-like conditions could be a result of the variation in those orchestrated conditions themselves. While it is clear that some crustaceans are not robust to forecasted changes in ocean pH, there is insufficient research to understand the scope of species' vulnerability nor the mechanisms underlying it.

For crustaceans in particular, warmer ocean temperature is associated with greater growth rates and molt frequencies (Hammond et al., 2006; Wang et al., 2006). When

warmer temperature is combined with ocean acidification, however, the potential effects may be exacerbated, mitigated, or unaffected (Kroeker et al., 2013). In the caridean shrimp, *Hippolyte californiensis*, for example, the combined effects of reduced pH and increased temperature resulted in increased growth in carapace length (Lowder et al., 2017), suggesting that reduced pH had no effect on growth. *Pandalus borealis* larvae experienced larger impacts on the speed of their development under increased temperature than from reduced pH alone, also demonstrating that increased temperature can elicit a greater biological response in comparison to reduced pH (Arnburg et al., 2011). In benthic juvenile lobster, *Hommarus gammarus*, increased temperature led to increased molt increment and increased mortality, but did not act synergistically with reduced pH (Small et al., 2016). Overall, research has shown that crustaceans vary on a species-specific level in response to experimentally reduced pH and increased temperature conditions and that experiment duration may influence a species response (Form & Riebesell, 2012). The complex and variable interactions between reduced pH and increased temperature, as well as the uncertainty surrounding responses to these changes, promote a need for longer term experiments on the interaction of both ocean acidification and ocean warming (Whiteley, 2011).

While growth and calcification are the primary metrics evaluated in ocean acidification and ocean warming research, there are other measures of health and reproduction that are important for commercially important species, yet rarely measured. In particular, two indices, the hepatosomatic index (HSI) and the gonadosomatic index (GSI), are commonly used to quantify development of the gonads and their proportion in

terms of body mass (López-Greco & Rodríguez, 1999; Sokolowicz et al., 2006). The hepatopancreas is the largest center of organic and inorganic reserves in crustaceans (Passano, 1960). Research has proposed that the HSI is depleted for energy utilization during reproductive cycles and the molt cycle (Beatty et al., 2005; Kyomo, 1988; Sokolowicz et al. 2006). Females of the prawn species *Macrobrachium olfersii* have been shown to have depleted HSI at the height of their GSI (Magalhaes et al., 2012). This demonstrates that there is an increased energy requirement of females during oocyte development, and this energy is provided by lipid and protein mobilization (Magalhaes et al., 2012). During the earlier stages (I-II) of ovarian development, there is a direct relationship between the indices, but in the later stage (III), the lowest values of GSI tend to correspond to the highest values of HSI (Magalhaes et al., 2012). This relationship was found in multiple species, including *Penaeus setiferus* and the crab *Neohelic granulata* (Castille & Lawrence, 1989; López-Greco & Rodríguez, 1999). In males and females, the fluctuation of HSI correlates with the molt cycle as well as food intake (Adiyodi, 1969; Kyomo 1988). The HSI reaches its maximum during the intermolt stage and is utilized during the molt for the lipid to sugar conversion necessary for the formation of chitin (Passano, 1960). Diversion of energy from growth and reproduction to compensatory responses, such as increased buffering of CO_2, has been proposed as an effect of medium-term exposure to reduced pH conditions (Whiteley, 2011). At the same time, experiments exploring the effects of increased temperature have shown increased feeding behavior, which is also associated with HSI values. Thus, we hypothesize that reduced pH and increased temperature have the ability to influence HSI and GSI values, or their

relationship to one another. A reduction or change in HSI or GSI could have far-reaching impacts on available energy stores and reproductive potential, both integral to survival and fitness.

Ancillary to adequate growth, survival and biomass accumulation is the ability to detect and consume prey. For crustaceans, food detection is accomplished by chemosensory sensilla, which are hair-like structures that house olfactory receptors (Schmitt & Ache, 1979). Aesthetascs are chemosensory sensilla present on the lateral filaments of crustacean antennules. A number of researchers have suggested that crustaceans flicking the lateral filaments of their antennules increase the contact of water with the aesthetascs, thereby enhancing the ability to "sniff" chemical stimuli present in seawater (Schmitt & Ache, 1979; Atema, 1985; Koehl, 1995). This behavior is known as "antennular flicking" and has been utilized as a proxy for chemoreception in crustacean species (Koehl, 2011).

Reduced pH conditions can negatively affect the chemosensory behavior of crustaceans (Dissanayake & Ishimatsu, 2011, Tierney & Aetma, 1986). For example, hermit crabs (*Pagurus bernhardus*) decrease the number of antennular flicks by 10 flicks (absolute value) in response to reduced pH (Kim et al., 2015). A reduction in antennular flicking would lead to less contact of aesthetascs with stimulants, which could have the potential to reduce reaction to stimuli. Although some studies like those on the deep-sea hermit crab, *Pagurus tanneri,* and the intertidal hermit crab, *Pagurus bernhardus,* found reduced antennular flicking in response to reduced pH, no such effects were observed in the lobster, *Panulirus argus* (Kim et al., 2015, de La Haye et al., 2011, Ross & Behringer,

2019). The mechanism for reduced pH impacting chemoreception is based in part on the potential morphological damage to the antenna, as observed in *Palaemon pacificus* (Kurihara et al., 2008), as well as the transformation of stimulants in changing seawater chemistry (Roggatz et al., 2016). Reduced pH conditions are known to affect chemoreception in several species of fish and may affect the olfactory receptor neurons of crustaceans as well (Leduc et al, 2013).

The range of potential and varied responses of crustaceans to ocean acidification and ocean warming conditions warrants further species-specific experiments. The ridgeback prawn, *Sicyonia ingentis*, is the largest species of sicyonid shrimp in the eastern Pacific, where it is commercially important throughout California and Mexico (Perez-Farfante, 1985) and supports a long-lived fishery in the Santa Barbara Channel. Due to its importance as a fished species and its future as a sustainable seafood option in southern California (McClenachan, 2014), there is motivation to examine how this species will respond to predicted changes in pH and temperature. As for other fished species, changing oceans have the potential to threaten catch potential through impacts on growth, time to reach life stages, and altering species geographic range.

Sicyonia ingentis (Burkenroad, 1938) is a benthic omnivore that is found from Monterey Bay, 36°50'N, 121°50'W, California, southward to Isla Maria Madre, 22°00'N, 106°16'W, Nayarit, Mexico, in the Gulf of California (Perez-Farfante & Booschthe 1981). It spans depths from 5-307 m, although it is most abundant in the range of 50 - 150 m (Stull et al., 1999). The abundance of *S. ingentis* decreases from its northern range

to its southern range, with major populations existing in areas with established fisheries (Perez-Farfante & Boothe 1981). The depth range that *S. ingentis* occupies exposes them to pH ranging from approximately 7.60 - 8.06 and a temperature range of approximately 9 - 14°C (Alin et al., 2012). Such natural environmental variation does not necessarily make crustacean species less vulnerable to ocean acidification and ocean warming conditions, as observed in porcelain crabs and barnacles (Paganini et al., 2014, Findlay et al., 2010). In this study, we exposed *S. ingentis* to reduced pH and increased temperature conditions for twelve weeks and measured their growth, survival, reproductive potential, energy storage, and chemosensory behavior.

METHODS

I. Animal Collection and Experimental Setup

Ridgeback prawn, *Sicyonia ingentis*, were collected in an otter trawl offshore San Diego, CA (32°39'N and 117°20'W) aboard the *RV Robert Gordon Sproul* and transported to the Scripps Institution of Oceanography (SIO), University of California, San Diego. Prawn were kept in communal tanks supplied with ambient flow-through seawater pumped from SIO pier (3-4 m depth, 300 m offshore) for three weeks prior to the start of the experiment. For the experiment, animals were transferred to an experimental setup that included four header tanks (150 L) that received filtered seawater pumped in from SIO pier, mixed via pumps, and fed flow-through seawater into 16 experimental tanks (2.8 L) housing an individual prawn. One header tank was maintained at ambient pH and temperature conditions (8.01 ± 0.02, 12.2 ± 0.8 °C), a second tank was adjusted for reduced pH (7.50 ± 0.02, 12.0 ± 0.7 °C), a third tank was adjusted for both reduced pH and increased temperature (7.50 ± 0.02, 16.2 ± 0.7 °C), and a fourth tank was adjusted for increased temperature (8.01 ± 0.03, 16.1 ± 0.5 °C). Experimental values were based on current predictions for a temperature increase of 4°C and a reduction of pH by 0.2 units by the year 2100 relative to the conditions that the species is currently experiencing in its environment at depth (IPCC, 2014).

Temperature conditions were achieved with adjusted flow of heated, chilled, and/or ambient seawater. Reduced pH conditions were attained through the bubbling of 100% CO_2 into the two pH adjusted header tanks and was controlled by a pH controller

(Apex Lite, pH accuracy 0.01; temperature accuracy 0.1°C; Neptune Systems, Morgan Hill, CA, USA) that continuously monitored pH and temperature. pH was reduced gradually over a period of 5 days prior to the experimental start day until target levels were met. All header tanks and individual aquariums were monitored daily for pH and temperature using a portable probe (Hach HQ40d, accuracy 0.01 pH, 0.1 C, CO, USA). In addition, water samples were collected from each header tank and a random experimental tank from each treatment in accordance with standard operating procedures (Dickson et al., 2007) and submitted to the Dickson laboratory for analysis of pH, total alkalinity, and density-based salinity. Results were run through CO2SYS to determine seawater pH. The difference between the Hach probe daily readings and the water samples was used to adjust daily readings to calculate means and standard deviations for all header and experimental tanks (Table 1). Prawn were held in experimental conditions for twelve weeks and fed a mixed diet of krill, squid, and food pellets (Sinking Carnivore Pellets, Kyorin, Himeji, Japan) 3 times per week.

Table 1: Temperature and carbonate chemistry parameters [mean ± s.d.] throughout the experiment. Mean is based on daily readings of experimental tanks for each treatment adjusted based on bottle sample readings. Sample size for pCO_2, salinity, TA, HCO_3, CO_3, Ω Ca, and Ω Ar is N = 2. Sample size for pHsws and temperature is N = 92.

	Treatment			
	Control	**Reduced pH**	**Increased T**	**Reduced pH/ Increased T**
pCO_2 (µatm)	386.1 ± 51.6	1439.8 ± 36.2	458.2 ± 26.3	1507.9 ± 13.9
pHsws	8.01 ± 0.03	7.50 ± 0.02	8.01 ± 0.03	7.50 ± 0.02
Temperature (°C)	12.2 ± 0.8	12.0 ± 0.8	16.0 ± 0.5	16.2 ± 0.7
Salinity (PSU)	33.55 ± 0.03	33.52 ± 0.01	33.55 ± 0.03	33.57 ± 0.01
TA (µmol/kgSW)	2206.7 ± 0.2	2207.5 ± 0.5	2208.0 ± 1.5	2207.5 ± 1.3
HCO_3 (µmol kg^{-1})	1876.1 ± 1.6	2096.4 ± 3.8	1877.0 ± 8.2	2084.8 ± 2.2
CO_3 (µmol kg^{-1})	132.5 ± 1.3	44.5 ± 1.5	133.3 ± 2.6	49.4 ± 0.4
Ω Ca	3.18 ± 0.04	1.07 ± 0.04	3.21 ± 0.06	1.19 ± 0.01
Ω Ar	2.03 ± 0.03	0.68 ± 0.02	2.06 ± 0.04	0.76 ± 0.01

II. Growth

Prior to the experiment and following each molt, animals were measured for carapace length (CL) to the nearest 0.01 mm, buoyant mass (in 500 mL seawater) and mass, both to the nearest 0.01 gram. Carapace length was measured from the posterior margin of the orbit to the middorsal posterior edge of the carapace and specimens ranged in CL from 20.11 to 27.18 mm, with a mean of 23.37 ±1.47 mm. Prawn were semi-randomly distributed into the four treatments based on CL, with the small number of females distributed evenly among treatments (see below). Prawn were monitored daily for molts and survival, with exuviae removed immediately. Sex was determined for each specimen

by the presence or absence of the male petasma. Five females were randomly assigned to each treatment for a female to male ratio of 5:11, with the exception of the reduced pH treatment that had a ratio of 6:10. Growth in CL, buoyant mass, and wet mass was measured as the percent change between the initial measurement and the first molt, between molt 1 and molt 2, and over the course of the experiment.

III. Dissections for HSI and GSI

All prawn still living at the end of the experiment were euthanized in a -20°C freezer before the gonads and hepatopancreas were dissected. For females, the ovarian stage was determined via macroscopic analysis based on the color, structure, and shape of the gonads (Dall et al., 1990). For males, the gonads included the testes and vas deferens. The wet weight of the gonads and hepatopancreas were measured separately for each prawn without any drying via a microbalance to the nearest 0.00001 gram (Sartorius CP225D Microbalance). Each prawn was also weighed to the nearest 0.00001 gram on the same scale prior to dissection. The same protocol was used for a selection of frozen specimens that died during the experiment. These data were used to calculate the gonadosomatic and hepatosomatic indices, GSI and HSI, respectively. GSI and HSI are defined as follows,

$$\text{GSI} = \frac{gonad\ wet\ weight}{body\ weight}\ x\ 100$$

$$\text{HSI} = \frac{hepatopancreas\ wet\ weight}{body\ weight}\ x\ 100$$

IV. Chemosensory Experiment

Prior to the end of the experiment, at 11 weeks, we examined the antennule flicking rates of prawn (a proxy for chemoreception) in response to a food stimulus. Prawn were starved for at least 48 hours prior to the two-day chemosensory experiment. Half of the prawn from each treatment were randomly selected to receive a control stimulus of water (1.5 mL) via a dropper positioned approximately 5 cm away from the antennules. This point at which the dropper was placed in the tank was marked on each container and the process was performed by one researcher throughout the experiment to ensure consistency. The other half of experimental prawn received a dose of mussel cue (1.5 mL) in the form of a mussel slurry diluted with their respective treatment water to a concentration of 0.5 g/L, which is above the known concentration to illicit antennule flicking in crustacean species (Zimmer-Faust and Case, 1983). Prawn were filmed for one minute prior to liquid insertion to establish a baseline antennule flicking rate using a handheld camera (Handicam HDR-CX405, Sony, New York, NY, USA) and for one minute following the liquid insertion. This process was repeated the second day of filming, with the stimulus liquids switched; prawn that received water the first day, received mussel cue the second day and vice versa. This protocol is similar to those of other crustacean chemosensory experiments (de La Haye et al., 2011; Kim et al., 2015 Ross & Behringer, 2019; Zimmer-Faust and Case, 1983)

Antennule flicks were counted from recorded videos for both right and left antennules for one minute before and one minute after liquid insertion. A flick was defined as a rapid downstroke of either antennule. Each video of flicking rates was

counted by two people without knowledge of which treatment prawn belonged to. Peculiar behavior, such as jumping back, was also noted.

V. Statistical Analysis

All statistics were performed in R v. 3.6.0 (R Core Development Team, 2019). Carapace length, body mass, and health indices (HSI and GSI) were each tested for normality using Shapiro-Wilk test and homogeneity using Levene's test (lawstat package; Gastwirth et al., 2019). Each metric was then compared across treatments using a one way ANOVA followed by a post-hoc Tukey's HSD when appropriate. Antennule flick counts after liquid insertion and relative to before liquid insertion were compared across treatments using a linear mixed effects model ANCOVA. Fixed factors were treatment and liquid added. Flicking rate prior to liquid insertion was a covariate. Individual prawn was added as a random effect. The model was fit with restricted maximum likelihood and the significance of fixed factors were evaluated via F-test using the nlme package (Pinheiro et al., 2018). Survival curves were created using Kaplan-Meier estimates and compared to expected values using a Mantel-Haenszel Test with the survminer and survival packages (Alboukadel et al., 2019; Therneau, 2015; Therneau & Grambsch, 2000). A relative risk analysis was performed to compare the relative risk of mortality across treatments (epitools package; Aragon, 2017). Data are presented as mean ± standard deviation.

RESULTS

Growth, Molting, and Survival:

Mortality began to occur at 8 weeks into the experiment and continued until the end of the experiment. Deaths occurred in all treatments and could not be attributed to any particular factor, however, they occurred primarily around the time when animals were molting for a second time. A total of 28 individuals died: 4 from the control, 4 from the reduced pH treatment, 11 from the reduced pH/increased temperature treatment, and 9 from the increased temperature treatment. The survival curves for each treatment were significantly different from each other (Fig. 1: Mantel-Haenszel Test, $\chi^2 = 9.4$, $p = 0.02$). The reduced pH/increased temperature treatment had a significantly higher relative risk of death compared to the control (Relative risk analysis; $p = 0.018$). The estimated relative risk of death in the reduced pH/increased treatment was 2.45 (145%) times the risk of death in the control, with a 95% confidence interval of 1.12 (12%) to 5.34 (434%) times.

All prawn molted during the experiment, with several individuals molting twice. Of the animals that molted twice, 4 were in the control, 4 in the reduced pH treatment, 8 in the increased temperature treatment, and 6 in the reduced pH/increased temperature treatment.

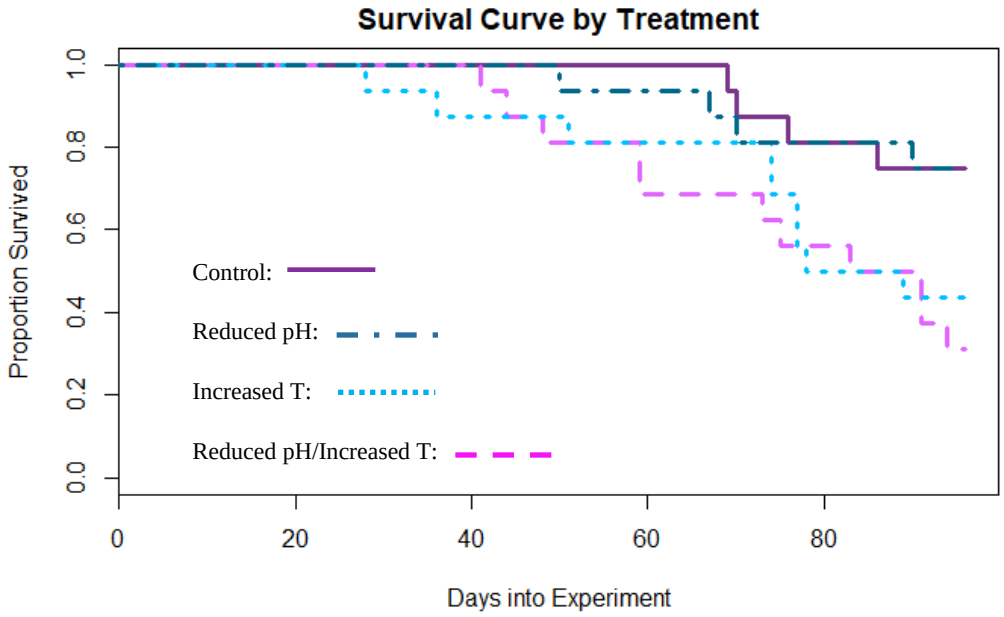

Figure 1: Survival curve using Kaplan-Meier estimates. Survival curves differed significantly between treatments (Mantel-Haenszel Test, $\chi^2 = 9.4$, $p = 0.02$). A relative risk analysis revealed strong evidence (p-value = 0.018) of a greater relative risk of death in the reduced pH/increased treatment relative to the control.

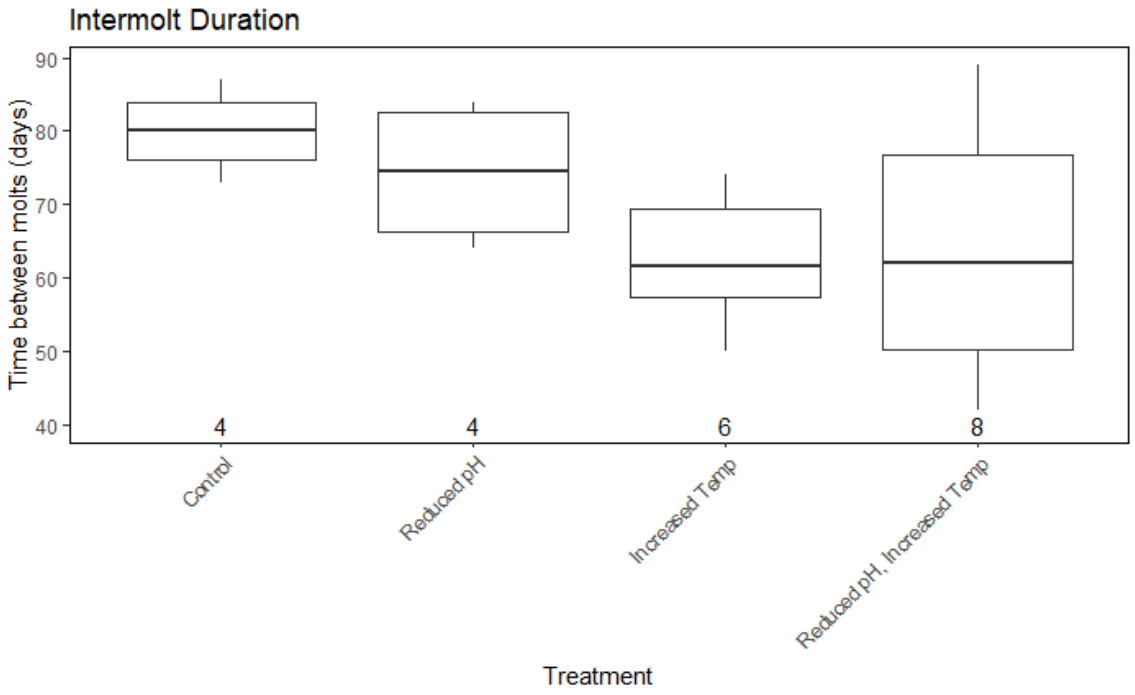

Figure 2: Mean time between Molt 1 and Molt 2 presented in days. Time between molt one and molt two did not differ between treatments (ANOVA: $F_{3,18} = 2.30$, $p = 0.11$). Box plot boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median. Black dots represent outliers that were included in the analyses. Numbers below boxes indicate sample size.

The average time between molt 1 and molt 2 was 80 ± 6 days (N = 4) for those in the control treatment, 74 ± 10 days (N = 4) for prawn in the reduced pH treatment, 63 ± 9 days (N = 6) for those in the increased temperature treatment, and 64 ± 17 days (N = 8) for those in the reduced pH/increased temperature treatment. These intermolt durations were not significantly different between treatments (ANOVA: $F_{3,18} = 2.30$, $p = 0.11$; Fig.

2)

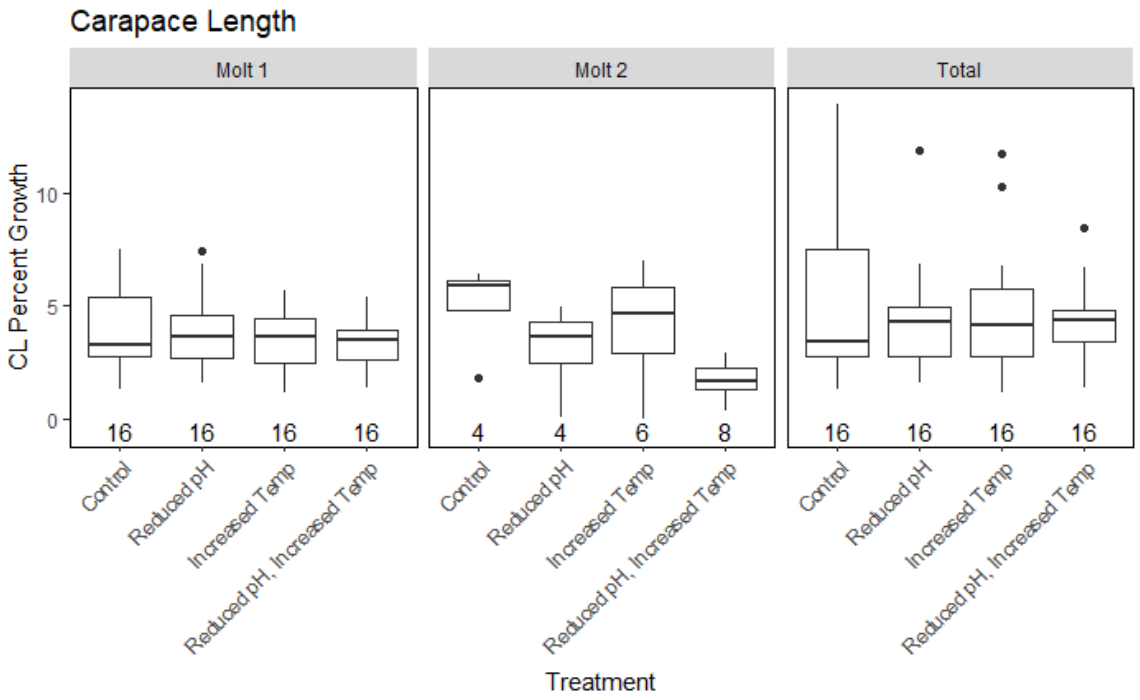

Figure 3: Mean percent growth in carapace length at Molt 1, Molt 2, and over the entire experiment. Treatment had a significant effect on percent CL growth at Molt 2 (ANOVA: $F_{3,18} = 3.36$, $p = 0.04$), with the reduced pH/increased temperature treatment having a significantly lower growth in comparison to the control (Tukey HSD). Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median. Black dots represent outliers for each treatment found to be non-influential due to Cook's Distance <0.5 for all analyses. Numbers below each box represent sample size.

All prawn exhibited an increase in carapace length by the end of the experiment (Fig. 3). There was no significant difference in percent growth of carapace length between treatments at the first molt, but there was a significant difference at the second molt (ANOVA: $F_{3,18} = 3.36$, $p = 0.04$). We saw a significant reduction in growth following the second molt in response to treatment. Running a Tukey HSD revealed that the reduced pH/increased temperature had significantly reduced growth in comparison to the control. When percent growth in carapace length over the entire experiment was

considered, however, there was no significant difference between treatments (ANOVA: $F_{3,60}$ = 0.34, p = 0.80). Prawn grew in CL an average of 5.21 ± 3.83% (N = 16) in the control treatment, 4.51 ± 2.44% (N = 16) in the reduced pH treatment, 4.27 ± 1.70 % (N = 16) in the reduced pH/increased temperature treatment, and 4.86 ± 2.84% (N = 16) in the increased temperature treatment.

There were no significant differences in body mass percent growth at either the first molt (ANOVA: $F_{3,28}$ = 0.56, p = 0.65; Fig. 4), the second molt (ANOVA: $F_{3,57}$ = 0.27, p = 0.85; Fig. 4), or over the duration of the experiment (ANOVA: $F_{3,16}$ = 3.17, p = 0.05; Fig. 4). All animals, except one that molted 2 days before the end of the experiment, experienced an increase in body mass throughout the duration of the experiment.

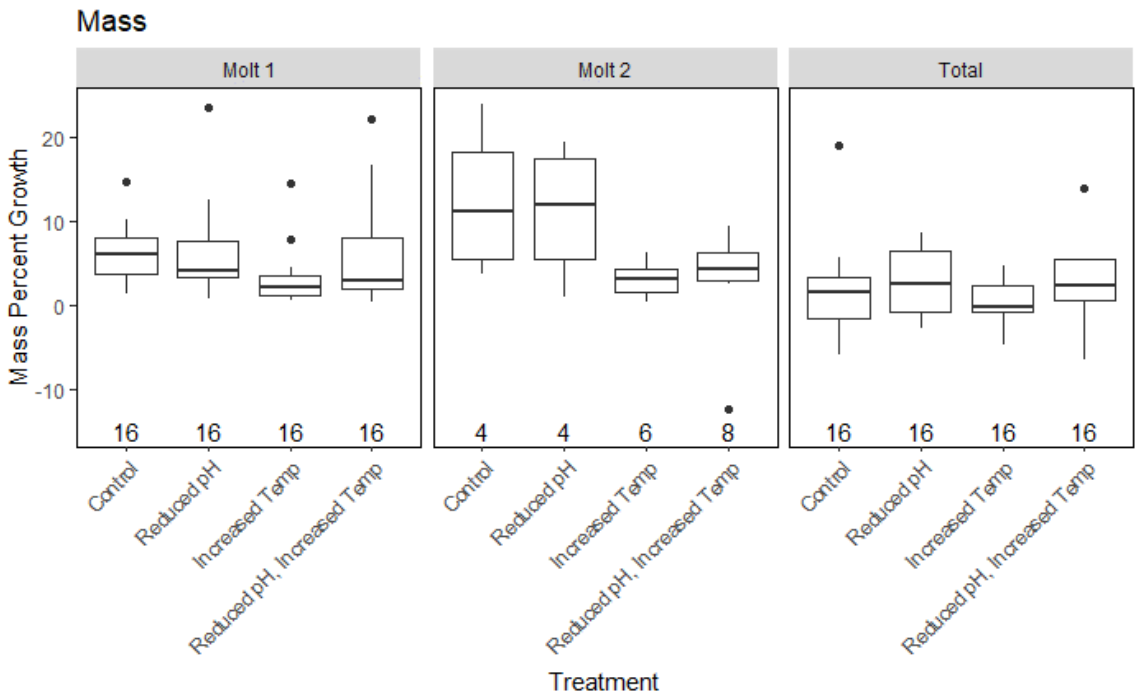

Figure 4: Percent body mass growth at Molt 1, Molt 2, and over the entire experiment. There were no significant differences between treatments at Molt 1, Molt 2, or for the duration of the experiment. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median. Black dots represent outliers for each treatment found to be non-influential due to Cook's Distance <0.5 for all analyses. Numbers below each box represent sample size.

Gonadosomatic Index:

All females (N = 16) dissected at the end of the experiment were in stage I for ovary development. Females had an average GSI of 1.18 ± 0.15% (N = 4) in the control treatment, 1.04 ± 0.41% (N = 4) in the reduced pH treatment, 1.00 ± 0.19% (N = 4) in the increased temperature treatment, and 1.25 ± 0.60% (N =4) in the reduced pH/increased temperature treatment. Males (N=42) had an average GSI of 0.94 ± 0.22% (N = 9) in the control treatment, 1.13 ± 0.39% (N = 9) in the reduced pH treatment, 0.93 ± 0.27% (N = 8) in the increased temperature treatment, and 1.05 ± 0.26% (N = 10) in the reduced

pH/increased temperature treatment. No significant effect of treatment was found for either female (ANOVA: $F_{3,13} = 0.39$, $p = 0.76$; Fig. 5) or male (ANOVA: $F_{3,33} = 1.81$, $p = 0.16$; Fig. 5) GSI.

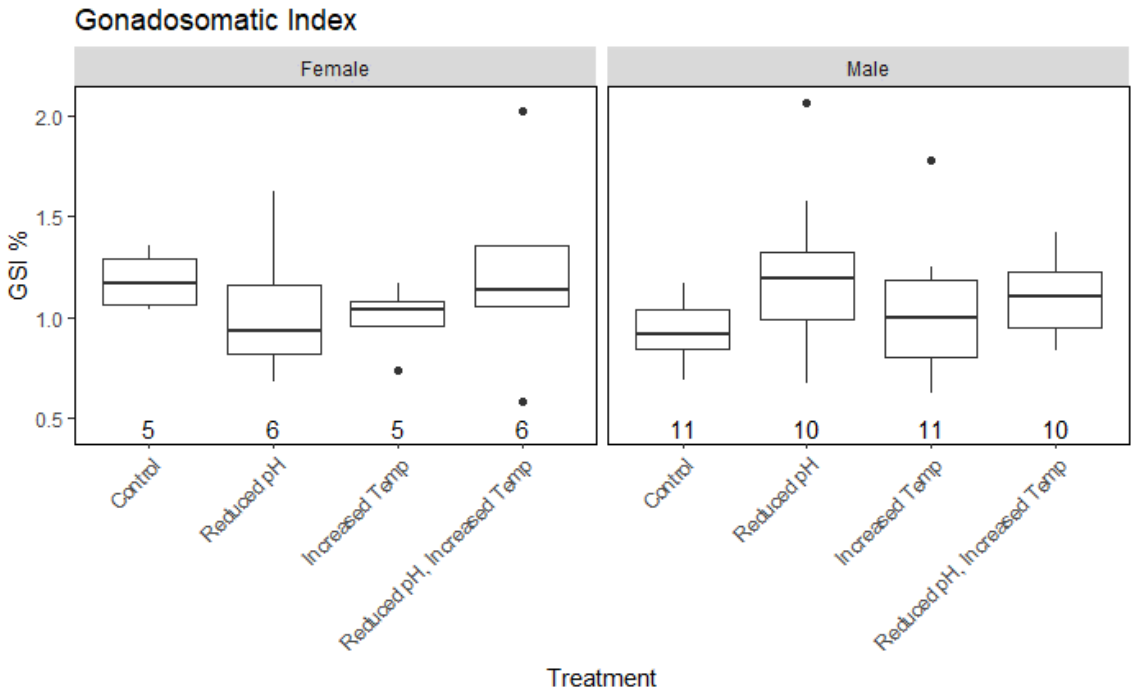

Figure 5: Mean gonadosomatic indices for females and males. No significant effect of treatment was found for female or male GSI. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median. Black dots represent outliers for each treatment found to be non-influential due to Cook's Distance <0.5 for all analyses. Numbers below each box represent sample size.

Hepatosomatic Index:

Mean male HSI was 1.60 ± 0.35% (N = 11) in the control treatment, 2.09 ± 0.70% (N = 10) in the reduced pH treatment, 1.17 ± 0.17% (N = 11) in the increased temperature treatment, and 1.68 ± 0.44% (N = 10) in the reduced pH/increased temperature treatment. Treatment had no significant effect on male HSI (ANOVA: $F_{3,33} = 2.73$, $p = 0.06$; Fig. 6). Mean female HSI was 1.60 ± 0.35% (N = 5) in the control, 2.09 ± 0.70% (N = 6) in the

reduced pH treatment, 1.17 ± 0.17% (N = 5) in the increased temperature treatment, and 1.68 ± 0.44% (N = 6) in the reduced pH/increased temperature treatment. Treatment had no significant effect on female HSI (ANOVA: $F_{[illegible]}$ = 2.73, p = 0.06; Fig. 6).

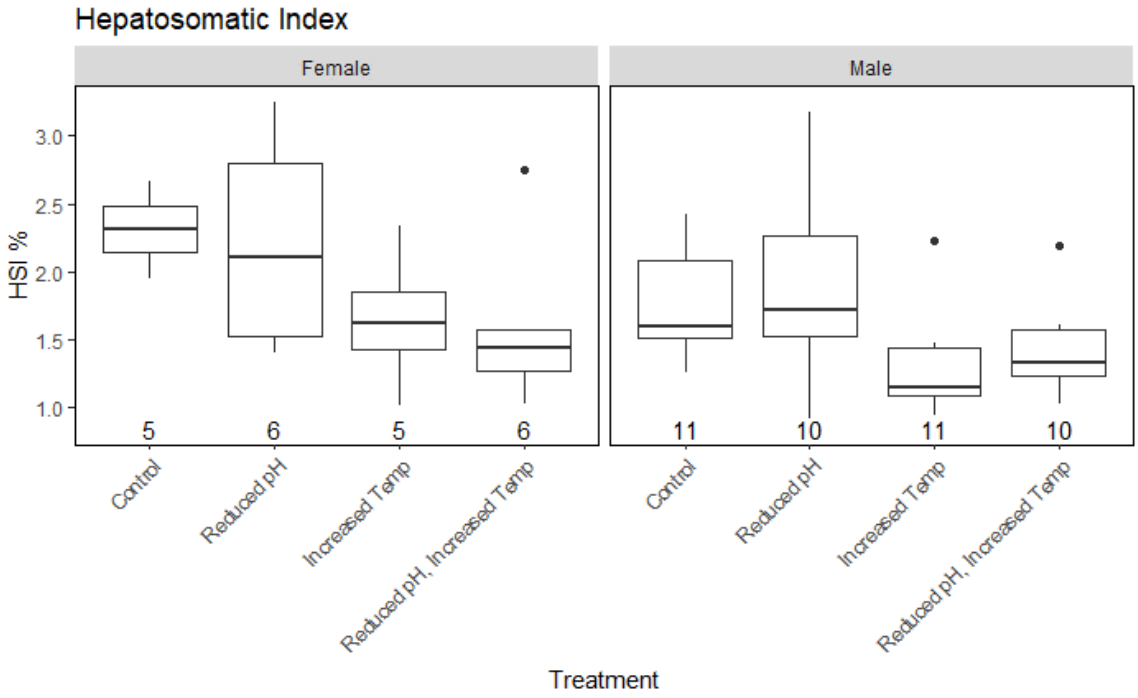

Figure 6: Mean hepatosomatic index for female and males. No significant effect of treatment was found for female or male his. Box boundaries = the 25th and 75th quartiles, error bars = 1.5 times the interquartile distance, center line = median. Black dots represent outliers for each treatment found to be non-influential due to Cook's Distance <0.5 for all analyses. Numbers below each box represent sample size.

Antennule Flicking rate:

We saw no effect of treatment on antennular flicking rate (Linear mixed effects model: $F_{[illegible]}$ = 1.39, p = 0.25; Fig. 7). There was strong evidence that the pre-flick rate is associated with the post-flick rate (F-value = 36.89, p-value < 0.001). For every increase in 1 pre-flick rate, there is an estimated increase of post-flick rate of 0.71 flicks. Flicking rates did not differ in response to the type of liquid that was inserted ($F_{[illegible]}$ = 1.39, p = 0.23;

Fig. 7), and therefore there was no effect of the mussel cue on antennular flicking. Despite showing no difference in antennule flicking rates, prawn exhibited different behaviors in response to the water and mussel cue. When given the mussel cue, prawn immediately began feeding behavior, which consisted of a sweeping movement of the chelae towards the mouthparts. Thus, the mussel cue was detected, but elicited feeding behavior rather than antennular flicking.

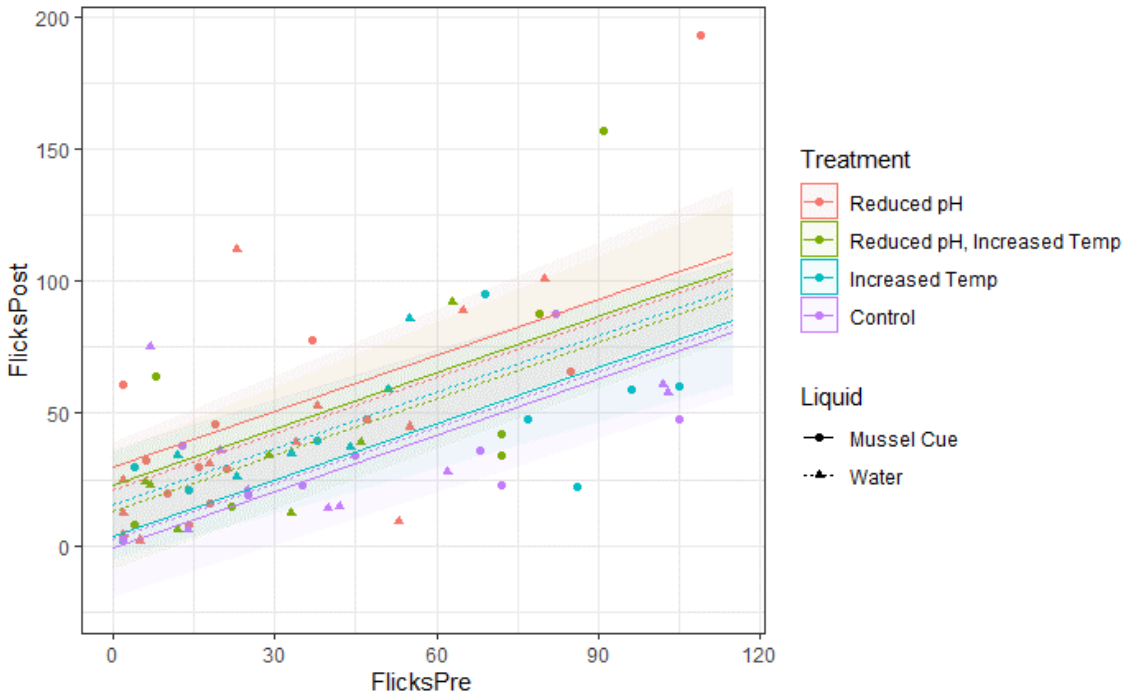

Figure 7: Linear mixed effects model of antennular flicking rates. Mussel cue is represented by circles and solid lines while the water stimulus is represented by triangles and dotted lines. There was no significant effect of treatment on antennular flicking rate. (Linear mixed effects model: $F_{7,28} = 1.39$, $p = 0.25$)

DISCUSSION

Ocean acidification and ocean warming pose a threat to marine organisms, including crustaceans. We studied the ridgeback prawn, *Sicyonia ingentis*, and found that it is robust to reduced pH in terms of survival, growth, health indices (GSI and HSI), and prey detection (antennular flicking). When reduced pH is combined with increased temperature, however, prawn experience reduced survival and reduced growth in carapace length following a second molt.

Mortality greater under combined stressors

Crustacean survival has been shown to be affected by both reduced pH (Kurihara et al., 2008) and increased temperature (Thomas et al., 2000) conditions. We found that the relative risk of mortality of the ridgeback prawn is significantly higher when reduced pH is combined with increased temperature, in comparison to ambient conditions. While the relative risk of mortality was similarly high in the increased temperature treatment, potentially reflecting a greater effect of warmer temperature, it was not statistically significant. Nonetheless, reduced pH alone appears to have no impact on the survival of *S. ingentis*, even after 12 weeks of exposure.

Our data suggest that there is a threshold temperature at which *S. ingentis* is no longer robust to the effects of reduced pH. A previous study on temperature sensitivity in *S. ingentis* revealed that the species could withstand a broad range in temperature, from 5-30°C, although prawn were only tested under these temperatures for a maximum of 24 hours (Herkelrath, 1977). In our experiment we used a temperature of 16°C, which is 4 degrees above the maximum for the depth at which they were collected. In these

conditions mortality did not occur until 8 weeks. Thus, for this population of *S. ingentis* there are consequences of prolonged exposure to higher than normal temperatures as well as a potential threshold for survival in warmer temperature conditions. Experiments using multiple temperature points in crustaceans have found that survival and growth can be largely unaffected by increasing temperatures until a specific threshold is reached (Thomas et al., 2000). Furthermore, reduced pH can decrease the thermal tolerance of crustacean species (Whiteley, 2011). This is a possible explanation of the trends we saw in survival. *S. ingentis* experienced reduced survival in response to a moderate increase of 3-4 degrees above their typical temperature at collection depth, but survival was only significantly reduced in comparison to the control in the group exposed to both increased temperature and reduced pH. The combined stressors together increased mortality in *S. ingentis*. Similar synergistic effects of reduced pH and increased temperature were observed in the red king crab (Swiney et al., 2017).

Crustacean molt frequency and intermolt duration are sensitive to temperature (Fowler, 1971) and reduced pH (Kroeker et al., 2011). Molt frequency and intermolt duration are increased due to the increased speed of the physiological processes of growth (Whiteley, 2011). In reduced pH, molt frequency and intermolt duration are decreased due to the decrease energy availability from maintaining acid-base regulation (Whiteley, 2011). We found that neither reduced pH, increased temperature, nor these stressors combined affected the intermolt duration of *S. ingentis*. All prawn molted at least once during the experiment, with the time between molts being lower on average, but not significant (possibly due to low sample size), for the two increased temperature

treatments. Though not significant, this pattern aligns with observations of other crustacean species that experience shorter intermolt durations when exposed increased water temperatures (Breteler, 1975; Fowler et al., 1971; Iguchi and Ikeda, 1995; Travis, 1954; Fowler et al., 1971; Breteler, 1975; Iguchi and Ikeda, 1995). Neither the pH nor the temperature conditions used in this experiment were sufficient to elicit changes in molt frequency over 3 months of exposure, however, this could be due to the low number of individuals that molted a second time. It may take longer exposure and a greater number of molts for changes in molt frequency to materialize, as found in other studies on crustaceans (Long et al., 2013).

Although the combined stressors of reduced pH and increased temperature did not affect intermolt duration, they did affect the second molt increment (CL), suggesting that they have additive, antagonistic effects on growth. Molt increment (CL) was 3.3% greater at the second molt for the control than the combined stressors treatment. This difference was evident even with the small number of prawn that molted a second time. When molt increment was considered over the duration of the experiment, this difference was masked by the greater number of animals that only molted once, thereby biasing the data towards the first molt. Other studies have shown that it can take multiple molt cycles to see the effects of reduced pH conditions on molt increment (Long et al., 2013). For example, when exposed to reduced pH conditions, tanner crabs experienced a reduced molt increment at the first molt, while king crabs took three molts for the effects to materialize (Long et al., 2013). Reduced molt increments at the second molt for *S. ingentis* exposed to reduced pH combined with increased temperature infers a loss in

growth that could be carried over due to inherent limits in molt increment, which naturally becomes lower with increased body size. Further, the reduced growth increment at the second molt may signal continued reductions in growth at subsequent molts. Both of these possibilities could have negative outcomes for maximum adult size reached during the short lifespan of these prawn.

Reduced linear growth, as measured by percent change in CL, in the combined stressor treatment was not matched by changes in body mass. Thus, in the combined stressor treatment, prawn increased their body mass by the same amount as other treatments. Another experiment on the combined effects of reduced pH and increased temperature saw an impact on body mass with no impact on CL (Swiney et al., 2017). Thus, we can see that combined stressors of reduced pH and increased temperature have the ability to affect only one aspect of growth in crustaceans. This motivates exploration of the difference between how these two growth mechanisms in crustaceans are impacted by environmental changes.

Health indices unaffected by reduced pH and increased temperature

Hepatosomatic (HSI) and Gonadosomatic (GSI) indices are used to quantify gonad development and energy storage. Several experiments have demonstrated the mobilization of the hepatopancreas reserves during gonadal development through variations of GSI and HSI (Beatty et al. 2005, Kyomo 1988, Sokolowicz et al. 2006). These indices relate to growth and the process of molting in crustaceans, because the energy reserves in the hepatopancreas are partially mobilized during the molt cycle (Adiyodi 1969, Kyomo 1988). Since the indices have been linked to growth, energy

storage, and feeding, we hypothesized that GSI and HSI, or their relationship to each other, could be affected by environmental conditions such as reduced pH and increased temperature. Neither index has been studied in response to the environmental conditions of reduced pH and increased temperature so far in shrimp and we found no effect of treatment on GSI or HSI for either sex.

The average GSI observed in females is consistent with the GSI values measured in a different species of prawn, *Metapenaeus joyneri* (Chu, 1995). The male GSI and female GSI of *S. ingentis* is this study were relatively similar to those for both *M. olfersii* and *M. joyneri*. The male HSI values of *S. ingentis* were variable and lower than those seen for both *M. olfersii* and *M. joyneri*. The female HSI values were comparable with the lower range of the *M. olfersii* for stage I ovarian development, but smaller than the values for *M. joyneri*. Regardless of treatment, the GSI and HSI indices of *S. ingentis* in our study were similar to those found for other species of prawn, indicating that the pH and temperature conditions used did not have an effect on the overall health of the species as measured by these indices.

These results may stem from the gonads not being at a late stage of development that would be sufficient to influence the mobilization of the hepatopancreas (Magalhães et al., 2012). There was a general trend of reduced HSI under treatments with increased temperature, but the values were not significantly different from the control. Due to the high mortality at the end of the experiment and our low female to male ratio, sample sizes were perhaps too small to detect a difference. This observed trend motivates more research with sufficient sample sizes to determine whether or not environmental

conditions have effects on these indices. Though the GSI and HSI indices are commonly used for evaluating crustacean health and reproductive potential in the fishing/aquaculture industry (Amtyas et al., 2013; Magalhães et al., 2012), these may not be the best metrics. Rather, determining the type and quantity of lipids present in the hepatopancreas may give better insight into whether or not animals have the necessary stored energy under changing environmental conditions. Our results suggest that GSI and HSI, as indicators of reproductive potential and energy storage, are not affected by the pH and temperature conditions used in our study.

Detecting prey in reduced pH and increased temperature conditions

The efficacy of crustacean prey detection mechanisms sometimes decreases under reduced pH conditions (de la Haye et al., 2011; Kim et al., 2015), including antennular flicking, which is an important mode of chemosensory behavior. While other studies have observed reductions in antennular flicking under reduced pH conditions (Tierney & Aetma, 1985), we observed no effect of treatment on the rate of antennular flicking in *S. ingentis*. A study on lobsters saw similar results (Ross & Behringer, 2019). Our observation may not guarantee that the species will not see changes prey detection under reduced pH conditions because our mussel cue also saw no effect on antennular flicking. Since our cue test did not work, it is not possible to connect a lack of difference between treatments with any certainty of no impact on antennular flicking.

While post-flick rate was strongly associated with pre-flick rate, it did not differ based on the type of liquid stimulus (water or mussel cue). This suggests that our mussel cue was insufficient to induce a response in antennular flick rate, but the mussel cue was

over a thousand times higher in concentration than needed for other crustacean responses. Thus, this was not likely the case. Regardless of the type of stimulus, antennular flick rate increased after a liquid was inserted. It is, therefore, possible that the prawn respond to incoming flow regardless of the chemical content to sense whether or not a cue is present. Yet, in most cases, prawn immediately engaged in feeding behavior after the insertion of the mussel cue. Feeding behavior is characterized by a sweeping movement of the chelae towards the mouthparts (Harpaz et al., 1987). An increase in antennular flicking may not be necessary to sense this concentrated cue so prawn immediately began feeding instead. Furthermore, the mussel cue is also a visual cue since it is not clear like the water. This could have resulted in visual rather than chemosensory detection of the mussel cue. Yet, this method of stimulating antennular flicking has been successful in other crustacean species (de la Haye et al., 2011; Kim et al., 2015; Ross & Behringer, 2019).

S. ingentis tolerant to pH outside natural range

Variation in species responses to reduced pH and increased temperature stressors similar in magnitude to those used in this study demonstrates the importance of experimental conditions. *S. ingentis* tolerated a pH of 7.50, which is 0.2 pH units lower than the pH values experienced in their natural range (Alin et al., 2012), without an observable effect of reduced pH alone on its survival, growth, health indices, or prey detection. The tolerance of *S. ingentis* could be a result of the length of the experiment, which lasted 12 weeks. It has been shown that in experiments of longer duration, specimens have time to acclimate to environmental conditions (Form & Riebesell, 2012). *S. ingentis* in the adult stage are generally offshore in deeper waters, which are more

stable in terms of pH. At the same time, in certain regions, such as the Santa Barbara channel, this species is present in shallower waters that could be more variable in pH (Anderson et al., 1985a). The depth range of this species is not certain, as some resources report that they are found down to 150 m, whereas others report depths to 325 m (Anderson et al., 1985b; López-Martínez et al., 2019; Perez-Farfante & Boothe, 1981). Within this depth range, *S. ingentis* likely experiences pH ranging from 7.60 – 8.06 (Alin et al., 2012). A majority of this species caught for study occurred in the range of 7.70-8.00 (50-150 m depth, according to Stull et al., 1999), which is the depth that our specimens were collected from. Robustness to pH values below an animal's natural range was also observed in other crustaceans, such as mantis shrimp (deVries et al., 2016). Some species of crustaceans possess physiological tolerance to pH conditions far outside the range they would experience in nature, and it is not clear why this is so. Those that are considered strong iono-regulators (Whiteley, 2011) are expected to be well-equipped to maintain the pH of their internal hemolymph, regardless of changes in external pH conditions. A deeper understanding of the physiological basis of species tolerance to environmental pH conditions is a critical component for establishing vulnerabilities to ocean acidification.

Combined stressors have greater effects

Ocean acidification and warming are occurring at the same time. These environmental stressors have the ability to intensify the effects of ocean acidification on organisms (Kroeker, 2013). For example, the negative effects of reduced pH on the growth rate of the sea star, *Asterias rubens*, are enhanced by increases in temperature

(Keppel et al., 2015). Studies exploring the combined effects of ocean acidification and warming on growth and survival of crustaceans have been mixed, with some studies revealing synergistic effects (Long et al., 2013) and others revealing no interactive effects (Lowder et al., 2017). The only effects observed in this study on *S. ingentis*, (higher mortality, lower second molt increment in CL) occurred in the reduced pH/increased temperature treatment. Unlike other studies where increased temperature compounds the already negative effects of reduced pH (Dissanayake & Ishimatsu, 2011; Swiney et al., 2017) our findings show that increased temperature is aggravated by reduced pH. Specifically, survival decreased in the reduced pH/increased temperature treatment only, but showed a similar, but not significant, trend in the increased temperature treatment. This suggests that *S. ingentis* is robust to reduced pH within a certain temperature range but becomes sensitive to pH outside that range. Thus, this species may be sensitive to the concurrent stressors of ocean acidification and warming. These results add to the growing knowledge base about the importance of combined stressors when evaluating and predicting species responses to ocean conditions associated with climate change.

Conclusions

S. ingentis was able to withstand 3 months of exposure to reduced pH outside its natural range without effects on its growth, reproductive potential, or antennular flicking (a proxy for prey detection). The resilience of *S. ingentis* to reduce pH exposure could be attributed to its geographic and depth range, strong physiological buffering capabilities, and/or a mobile lifestyle. Yet, increased temperature in combination with reduced pH caused a relatively significant reduction in the second molt increment (CL) as well as a

significant increase in mortality in only 12 weeks of exposure. These results point to the importance of multi-stressor effects on species vulnerability. This species seems to be able to withstand a significant decrease in pH values without detectable sublethal effects, but this robustness might be compromised when temperature is simultaneously increased. Under the reduced pH and increased temperature parameters used in this study, *S. ingentis* experiences negative impacts on survival and the possibility of reduced growth. These experimental results provide insights into how *S. ingentis* may respond to changing ocean conditions as well as information useful for establishing this species as an underutilized fishery in the future.

REFERENCES

Adiyodi, R. T. 1969. On the storage and mobilization of organic resources in the hepatopancreas of a crab *Paratelphusa hydrodromus*. Experientia, 25: 43-44.

Alin, S. R., Feely, R. A., Dickson A. G., Hernández-Ayón, M., Juranek, L. W., Ohman, M. D. & Goericke, R. 2012. Robust empirical relationships for estimating the carbonate system in the southern California Current System and application to CalCOFI hydrographic cruise data (2005–2011). Journal of Geophysical Research, Vol. 117, C05033.

Amtyaz, K. M. A., Khan M. Z., & Hashmi M. U. A. 2013. Studies on Gonadosomatic Index & Stages of Gonadal Development of Stripped Piggy Fish, *Pomadasys stridens* (Forsskal, 1775) (Family: Pomadasyidae) of Karachi Coast, Pakistan. JEZS;.1(5):3-28.

Anderson, S. L., Botsford, L. W., & Clark, W. H. J. 1985a. Size Distributions and Sex Ratios of Ridgeback Prawns (*Sicyonia ingentis*) in the Santa Barbara Channel (1979-1981). CalCOFI, XXVI (Figure 1), 169–174.

Anderson, S. L., Wallis, C. H., Chang, E. S., & Chang, J. 1985b. Multiple spawning and molt synchrony in a free spawning shrimp (*Sicyonja ingentis*: penaeoidea). Biological Bulletin

Anthony, K. R. N., Kline, D. I., Diaz-Pulido, G., Dove, S., Hoegh-Guldberg, O. 2008. Ocean acidification causes bleaching and productivity loss in coral reef builders. Proceedings of the National Academy of Sciences. 105 (45) 17442-17446.

Aragon, T. A. 2017. Epitools. Epidemiology Tools. R package version 0.5-10. https://CRAN.R-project.org/package=epitools

Arnberg, M., Calosi, P., Spicer, J. I., Tandberg, A. H. S., Nilsen, M., Westerlund, S., & Bechmann, R. K. 2013. Elevated temperature elicits greater effects than decreased pH on the development, feeding and metabolism of northern shrimp (*Pandalus borealis)* larvae. Marine Biology, 160(8), 2037–2048. https://doi.org/10.1007/s00227-012-2072-9.

Beatty, S. J., Morgan, D. L. &. Gill, H. S. 2005. Life history and reproductive biology of the gilgie, *Cherax quinquecarinatus*, a freshwater crayfish endemic to southwestern Australia. Journal of Crustacean Biology, 25(2): 251- 262.

Bobelmann, F., Romano, P., Fabritius, H., Raabe, D. & Epple, M. 2007. The composition of the exoskeleton of two crustacea: the American lobster *Homarus americanus* and the edible crab *Cancer pagurus*. Thermochim. Acta 463, 65–68.

Breteler, W. K. 1975. Laboratory experiments on the influence of environmental factors on the frequency of moulting and the increase in size at moulting of juvenile shore crabs, *Carcinus maenas*. Netherlands Journal of Sea Research, 9: 100–120.

Castille, F. L., & Lawrence, A. L. 1989. Relationship between maturation and biochemical composition of the gonads and digestive glands of the shrimps *Penaeus aztecus* Ives and *Penaeus setiferus* (L.). Journal of Crustacean Biology, 9(2): 202-211.

Chu, K. H. 1995. Aspects of reproductive biology of the shrimp *Metapenaeus joyneri* from the Zhujiang estuary, China. Journal of Crustacean Biology 15: 214–219.

Dall, W., B. J. Hill, P. C. Rothlisberg, & D. J. Starples. 1990. The biology of the Penaeidae. Pp. 1–461 in J. H. S. Blaster and A. J. Southward, eds. Advances in Marine Biology, Vol. 27. Academic Press, San Diego.

deVries, M. S., deVries, M. S., Webb, S. J., Tu, J., Cory, E., Morgan, V., & Sah, R. L. 2016. Stress physiology and weapon integrity of intertidal mantis shrimp under future ocean conditions. Scientific Reports, 6: 38637.

Dickson, A. G., Sabine, C. L., and Christian, J. R. 2007. Guide to Best Practices for Ocean CO2 Measurements. North Pacific Marine Science Organization, Sidney, BC. 191 pp.

Dissanayake A., Ishimatsu, A. 2011. Synergistic effects of elevated CO2 and temperature on the metabolic scope and activity in a shallow-water coastal decapod (*Metapenaeus joyneri*; Crustacea: Penaeidae). ICES J Mar Sci 68:1147–1154.

Fernandez, C. H. 1995. Chemoreception studies in relation to feeding responses in the marine shrimps *Penaeus indicus* H. Milne Edwards and *Metapenaeus dobsoni* Miers. Cochin University of Science and Technology.

Findlay, H. S., Kendall, M. A., Spicer, J. I. & Widdicombe, S. 2010. Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. Estuar. Coast. Shelf Sci. 86, 675–682.

Form, A. U. & Riesebell, U. 2012. Acclimation to ocean acidification during long-term CO_2 exposure in the cold-water coral *Lophelia pertusa.* Glob. Change Bio. 18, 843–853.

Fowler, S., Small, L., and Keckecs, S. 1971. Effects of temperature and size on molting of euphausiid crustaceans. Marine Biology, 11: 45–51.

Frey, H. W. 1971. California's living marine resources and their utilization. Calif. Dep. Fish Game, Resour. Agency, 148 p

Gastwirth, J. L., Gel, Y. R., Hui, W. L., Lyubchich V., Miao, W., & Noguchi, K. 2019. lawstat: Tools for Biostatistics, Public Policy, and Law. R package version 3.3. https://CRAN.R-project.org/package=lawstat

Hammond, K. S., Hollows, J. W., Townsend, C. R., & Lokman, P. M. 2006. Effects of temperature and water calcium concentration on growth, survival and moulting of freshwater crayfish, *Paranephrops zealandicus*. Aquaculture, 251, pp. 271-279

Harpaz, D., Kahan, R., Galun, I. 1987. Responses of freshwater prawn, Macrobrachium rosenbergii, to chemical attractants. Moore Chem. Ecol. 13:9. 1957–1965.

Hartnoll, R. G. 2001. Growth in Crustacea: Twenty years on. Hydrobiologia 449**,** 111–122.

Herkelrath, J. M. 1977. Some aspects of the growth and temperature tolerance of the ridgeback prawn, *Sicyonia ingentis* Burkenroad in Southern California waters. M.S. Thesis, Whittier College, 67 pp.

Iguchi, N. & Ikeda, T. 1995. Growth, metabolism and growth efficiency of a euphausiid crustacean *Euphausia pacifica* in the southern Japan Sea, as influenced by temperature. Journal of Plankton Research, 17: 1757–1769.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Eds. by Core Writing Team, R. K. Pachauri and L. A. Meyer. IPCC, Geneva, Switzerland, 151 pp.

Kassambara, A., Kosinski, M., & Biecek, P. 2019. survminer: Drawing Survival Curves using 'ggplot2'. R package version 0.4.6. https://CRAN.R-project.org/package=survminer

Keppel, E. A., Scrosati, R. A. & Courtenay, S. C. 2015. Interactive effects of ocean acidification and warming on subtidal mussels and sea stars from Atlantic Canada. Mar. Biol. Res. 11**,** 337–348.

Kim, T. W., Taylor, J., Lovera, C., Barry, J. P. 2015. CO2-driven decrease in pH disrupts olfactory behaviour and increases individual variation in deep-sea hermit crabs. ICES Journal of Marine Science, doi:10.1093/icesjms/fsv019.

Koehl, M. A. R. 1995. Fluid flow through hair-bearing appendages: feeding, smelling, and swimming at low and intermediate Reynolds number. In: Ellington CP, Pedley TJ (eds) Biological fluid dynamics. Soc Exp Biol Symp 49, pp 157–182.

Koehl, M. A. R. 2010. Hydrodynamics of Sniffing by Crustaceans. In: Breithaupt T., Thiel M. (eds) Chemical Communication in Crustaceans. Springer, New York, NY.

Kroeker, K. J., Kordas, R. L., Crim, R. N., Singh, G. G. 2010. Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. Ecology Letters, 13, 1419–1434.

Kroeker, K. J., Micheli, F., Gambi, M. C., Martz, T. R. 2011. Divergent ecosystem responses within a benthic marine community to ocean acidification. Proceedings of the National Academy of Sciences of the United States of America, 108, 14515–14520.

Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology, 19: 1884–1896.

Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M. & Ishimatsu, A. 2008. Long-term effects of predicted future seawater CO2 conditions on the survival and growth of the marine shrimp *Palaemon pacificus*. J. Exp. Mar. Biol. Ecol. 367, 41–46.

Kyomo, J. 1988. Analysis of the relationship between gonads and hepatopancreas in males and females of the crab *Sesarma intermedia*, with reference to resource use and reproduction. Marine Biology, 97(1): 87-93.

Leduc, A. O., Munday, P. L., Brown, G. E. & Ferrari, M. C. 2013. Effects of acidification on olfactory-mediated behaviour in freshwater and marine ecosystems: a synthesis. Phil Trans R Soc B Biol Sci. 368.

Long, W. C., Swiney, K. M., Harris, C., Page, H. N. & Foy, R. J. 2013 Effects of ocean acidification on juvenile red king crab (*Paralithodes camtschaticus*) and tanner crab (*Chionoecetes bairdi*) growth, condition, calcification, and survival. PLoS One 8, e60959.

Magalhães, T.Mossolin, E.C., Mantelatto, F.L. 2012. Gonadosomatic and hepatosomatic indexes of the freshwater shrimp *Macrobrachium olfersii* (Decapoda, Palaemonidae) from São Sebastião island, southeastern Brazil. Panam. J. Aquat. Sci.71-9.

McClenachan, L., Neaty, B. P., Al-Abdulrazzak, D., Witkin, T. Taylor, Fisher, K., Kittinger, J. N. 2014. Do community supported fisheries (CSFs) improve sustainability? Fisheries Research, Volume 157. 62-69.

McCulloch, M., Falter, J., Trotter, J. 2012. Coral resilience to ocean acidification and global warming through pH up-regulation. Nature Clim Change 2, 623–627.

Paganini, A. W., Miller, N. A. & Stillman, J. H. 2014. Temperature and acidification variability reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes cinctipes*. J. Exp. Biol. 217**,** 3974–3980.

Perez-Farfante, I. & Boothe, B. B. 1981. *Sicyonia martini*, a new rock shrimp (Decapoda: Penaeoidea) from the American Pacific. J. Crustacean Bio I. 1:424-432.

Perez-Farfante, I. 1985. The rock shrimp genus *Sicyonia* (Crustacea: Decapoda: Penaeoidea) in the eastern Pacific. Fishery Bulletin 83:1-79.

Pillay, K. K. & Nair, N. B. 1973. Observations on the biochemical changes in gonads and other organs of *Uca annulipes, Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) during the reproductive cycle. Marine Biology, 18(3): 167-198.

Pinheiro, J., Bates, D., DebRoy, S., Sarkar, D. & R Core Team. 2018. nlme: Linear and Nonlinear Mixed Effects Models_. R package version 3.1-137, <URL: https://CRAN.R-project.org/package=nlme>.

Pörtner, H. O., 2008. Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217

R Core Team 2019. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Ries, J. B., Cohen, A. L. & McCorkle, D. C., 2009. Marine calcifiers exhibit mixed responses to CO2-induced ocean acidification. Geology 37, 1131–1134.

Ries, J. B., 2011. A physicochemical framework for interpreting the biological calcification response to CO2-induced ocean acidification. Geochim. Cosmochim. Ac. 75**,** 4053–4064.

Roggatz, C. C., Lorchi, M. Hardege, J. D., Benoit, D. M. 2016. Ocean acidification affects marine chemical communication by changing structure and function of peptide signalling molecules. Glob. Change Bio. 22, 3914–3926.

Roleda, M. Y., Boyd, P. W. & Hurd, C. L. 2012. Before ocean acidification: califier chemistry lessons. J. Phycol. 48**,** 840–843.

Rosa, R. & Nunes, M. L. 2003. Changes in organ indices and lipid dynamics during the reproductive cycle of *Aristeus antennatus*, *Parapenaeus longirostris* and *Nephrops norvegicus* (Decapoda) from the Portuguese south coast. Crustaceana, 75(9): 1095-1105.

Ross, E., Behringer, D. 2019. Changes in temperature, pH, and salinity affect the sheltering responses of Caribbean spiny lobsters to chemosensory cues. Sci. Rep. 9. 4375.

Small, D. P., Calosi, P., Boothroyd, D., Widdicombe, S. & Spicer, J. I. 2016. The sensitivity of the early benthic juvenile stage of the European lobster Homarus Gammarus (L.) to elevated pCO2 and temperature. Mar. Bio. 163: 53.

Schmitt B. C. & Ache, B. W. 1979. Olfaction: responses of a decapod crustacean are enhanced by flicking. Science 205:204–206.

Sokolowicz, C. C., Bond-Buckup, G. & Buckup, L. 2006. Dynamics of gonadal development of *Aegla platensis* Schmitt (Decapoda, Anomura, Aeglidae). Revista Brasileira de Zoologia, 23(4): 1153-1158.

Stull, J. K., Allen, M. J., Moore, S. L., Tang, C. L. 1999. Relative abundance and health of megabenthic invertebrate species on the southern California shelf in 1994. In: Weisberg SB, Elmore D (eds) Southern California coastal water research project annual report 1999–2000. Southern California Coastal Water Research Project, Westminster, pp 189–209

Swiney, K. M., Long, C. W., Foy, R. J. 2017. Decreased pH and increased temperatures affect young-of-the-year red king crab (*Paralithodes camtschaticus*). ICES Journal of Marine Science. 74:4. 1191–1200.

Taylor, J. R. A., Gilleard, J. M., Allen, M. C., and Deheyn, D. D. 2015. Effects of CO2-induced pH reduction on the exoskeleton structure and biophotonic properties of the shrimp *Lysmata californica*. Scientific Reports, 5: 10608.

Therneau, T. M. 2015. A Package for Survival Analysis in S. version 2.38, URL: https://CRAN.R-project.org/package=survival.

Therneau, T. M. & Grambsch, P. M. 2000. Modeling Survival Data: Extending the Cox Model. Springer, New York. ISBN 0-387-98784-3.

Thomas, C., Crear, B. & Hart, P. 2000. The effect of temperature on survival, growth, feeding and metabolic activity of the southern rock lobster, *Jasus edwardsii*. Aquaculture, 185(1–2), 73–84. https://doi.org/10.1016/S0044-8486(99)00341-5

Tierney, A. J. & Atema, J. 1986. Effects of acidification on the behavioral response of crayfishes (*Orconectes virilis* and *Procambarus acutus*) to chemical stimuli. Aquatic Toxicology, 9(1), 1–11. https://doi.org/10.1016/0166-445X(86)90002-0

Travis, D. F. 1954. The molting cycle of the spiny lobster, *Panulirusargus Latreille*. I. Molting and growth in laboratory-maintained individuals. The Biological Bulletin, 107: 433–450.

Wang, W. N., Wang, A. L., Yuan, L., Xiu, J., Liu, Z. B. & Sun, R. Y. 2006. Effects of temperature on growth, adenosine phosphates, ATPase and cellular defense response of juvenile shrimp *Macrobrachium nipponense*. Aquaculture, 256, pp. 624-630

Wickins, J. F. 1984. The effect of hypercapnic sea water on growth and mineralization in penaied prawns. Aquaculture 41**,** 37–48.

Zimmer-Faust, R. K., Case, J. F. 1983. A proposed dual role of odor in foraging by the California spiny lobster, Panulirus interruptus (Randall). Biological Bulletin. 164: 341-53.