Warming and pCO_2 effects on Florida stone crab larvae

*Philip M. Gravinese[a,b], Ian C. Enochs[c,d], Derek P. Manzello[d], and Robert van Woesik[a]

[a]Florida Institute of Technology, Department of Biological Sciences, 150 W. Univ. Blvd. Melbourne, FL 32901, United States of America, rvw@fit.edu

[b]Mote Marine Laboratory, Fisheries Ecology and Enhancement, 1600 Ken Thompson Way, Sarasota, FL 34236, United States of America, pgravinese@mote.org

[c]Cooperative Institute for Marine and Atmospheric Studies, Rosenstiel School of Marine and Atmospheric Science, University of Miami, Miami, Florida, Biscayne, FL 33149, United States of America, ian.enochs@noaa.gov

[d]Atlantic Oceanographic and Meteorological Laboratories, National Oceanic and Atmospheric Administration, 4301 Rickenbacker Causeway, Miami, Florida, 33149, United States of America, derek.manzello@noaa.gov

*Corresponding author: pgravinese@mote.org

Phone: 941-388-4441

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Running Head: Effects of Temperature and OA on larval stone crabs

Abstract

Greenhouse gas emissions are increasing ocean temperatures and the partial pressure of CO_2 (pCO_2), resulting in more acidic waters. It is presently unknown how elevated temperature and pCO_2 will influence the early life history stages of the majority of marine coastal species. We investigated the combined effect of elevated temperature (30°C control and 32°C treatment) and elevated pCO_2 (450 µatm control and 1100 µatm treatment) on the (i) growth, (ii) survival, (iii) condition, and (iv) morphology of larvae of the commercially important Florida stone crab, *Menippe mercenaria*. At elevated temperature, larvae exhibited a significantly shorter molt stage, and elevated pCO_2 caused stage-V larvae to delay metamorphosis to post-larvae. On average, elevated pCO_2 resulted in a 37% decrease in survivorship relative to the control; however the effect of elevated temperature reduced larval survivorship by 71%. Exposure to both elevated temperature and pCO_2 reduced larval survivorship by 80% relative to the control. Despite this, no significant differences were detected in the condition or morphology of stone crab larvae when subjected to elevated temperature and pCO_2 treatments. Although elevated pCO_2 could result in a reduction in larval supply, future increases in seawater temperatures are even more likely to threaten the future sustainability of the stone-crab fishery.

1. Introduction

At the current rate of fossil-fuel emissions, the partial pressure of CO_2 in seawater (pCO_2) is expected to increase from 400 µatm to 700–1000 µatm by the year 2100 (IPCC, 2013), resulting in a decrease in pH of 0.41 units. This process is often referred to as ocean acidification (Caldeira and Wickett, 2003). In addition, many coastal marine habitats are experiencing an accelerated rate of change in carbonate chemistry because of increased urbanization, coastal development, and wetland degradation (Bauer et al., 2013). Such activities are increasing nutrient-rich runoff, which when coupled with the degradation of organic material can cause elevated seawater pCO_2 events in coastal habitats (Bauer et al., 2013; Melzner et al., 2013; Ekstrom et al., 2013; Wallace et al., 2014). As a result, some coastal ecosystems are already experiencing conditions that either exceed critical thresholds for organisms, or have moved outside the range of normal pH conditions (Hauri et al., 2013; Harris et al., 2013). Increasing atmospheric CO_2 also simultaneously warms the oceans. By 2100, the ocean temperatures are expected to increase by 2–4°C (IPCC, 2013). The combined effect of anthropogenic CO_2 and elevated ocean temperature will pose challenges for less tolerant marine organisms, resulting in local extinction of numerous marine species and changes in global distribution patterns (et al., 2005).

Single-stressor studies on the tolerances of marine crustaceans to elevated pCO_2 have resulted in variable responses (i.e., positive, negative, mixed, and sometimes neutral), which also depend on the geographic location of the population (Walther et al., 2010) and the taxa studied (Ries et al., 2009; Kroeker et al., 2013). Many populations living in intertidal and coastal habitats, which experience frequent and extreme fluctuations in seawater physico-chemical factors, are thought to have the physiological and behavioral mechanisms necessary to tolerate future seawater changes

(Widdicombe and Spicer, 2008; Melzner et al., 2009; Whiteley, 2011; Byrne, 2011). Early life-history stages can, however, exhibit more sensitivity to changing environmental conditions than adult conspecifics (Whiteley, 2011). For example, acidified seawater did not alter metabolic rates in the adult green porcelain crab *Petrolisthes cinctipes* (Paganini et al., 2014), however the conspecific embryos exhibited slower metabolic rates under the same treatment (Carter et al., 2013). Similarly, juvenile porcelain crabs showed reduced survivorship when exposed to elevated pCO_2 conditions (Ceballos-Osuna et al., 2013). The varying sensitivity of crustacean life stages to elevated pCO_2 is likely the result of their ability to regulate blood hemolymph (i.e., the acid-base balance), which can disrupt enzymes and hormones that are necessary for molting, and can lead to abnormalities, including reduced body size (mass), calcification and morphological deformities (Kurihara et al., 2008; Arnold et al., 2009; Walther et al., 2010; Coffey et al., 2017). These CO_2-associated morphological changes may in turn negatively impact larval survival by altering swimming behaviors, including the ability to regulate buoyancy, maintain vertical position, and avoid predators (Sulkin, 1984; Morgan, 1989).

Temperature is one of the most critical environmental factors that can impact larval survival, molt stage duration, and development of crustaceans (Costlow et al. 1960, Naylor 1965). The impact of elevated seawater pCO_2 on crustaceans may become even more extreme in the context of ocean warming, as elevated temperature accelerates metabolism, and destabilizes proteins (Costlow and Bookhout, 1971; , 2008; Byrne, 2011). Additionally, extreme temperatures limit oxygen supply (et al., 2006), which can impact metabolism, and eventually lead to acidosis (Rahn, 1966; Rastrick et al., 2014). The effects of acidosis can be intensified under elevated seawater pCO_2, leading to hypercapnia and the impairment of oxygen transport systems (and Farrell, 2008; Melzner et al., 2013). Increases in temperature significantly affect

some crustacean larvae (i.e., *Sesarma*, *Callinectes*, *Menippe* spp.) by shortening molt-stage durations, reducing survivorship, and resulting in smaller individuals (Costlow et al., 1960; Ong and Costlow, 1970; Leffler, 1972). For example, early stage *C. sapidus* larvae exhibited a 15% decrease in survivorship when exposed to increased temperatures (Costlow and Bookhout 1960). Reductions in size under elevated temperatures are the result of individuals passing through larval development too quickly to accumulate sufficient lipid reserves to sustain additional growth (Swingle et al. 2013). Furthermore, certain enzymes within crustacean larvae may only be active at certain temperatures, and at elevated temperatures these pathways may be not operating efficiently (Costlow and Bookhout 1971). Therefore, determining both the effects of elevated temperature and pCO_2 on early life stages of crustaceans are necessary to realistically determine species responses to conditions projected by the end of the century. Understanding the influences of such environmental changes is particularly relevant for fisheries species.

The stone crab, *Menippe mercenaria*, contributes ~$30 million a year to Florida's economy (Florida Fish and Wildlife Conservation Commission Stock Assessments, 1998–2016). From 1998–2016, the mean annual commercial catch has declined from 3.5 to 2.7 million pounds of claws per year (Florida Fish and Wildlife Conservation Commission Stock Assessments 1998–2016). Much of the stone crab life-cycle, including embryonic development, larval release, and post-larval recruitment, occurs within coastal regions (Lindberg and Marshall, 1984; Krimsky and Epifanio, 2008; Krimsky et al., 2009; Gandy et al., 2010). These coastal habitats are also threatened by local human activities. Land-use change along parts of Florida's coastline is resulting in nutrient-rich runoff, which will likely amplify nearshore acidification (Bauer et al., 2013) and influence all coastal marine life. Despite living in environments that experience fluctuations in carbonate chemistry, part of the stone crab's life cycle shows sensitivity to

seawater acidification. For example, stone crab embryonic development is slower and hatching success is reduced when embryos are exposed to lower ocean pH (Gravinese, 2018). Therefore, it is possible that other components of their life-cycle may also be sensitive. We tested the hypotheses that elevated pCO_2 (~400 and 1100 μatm), elevated temperature (30°C and 32°C), and their combined effect results in reduced survivorship of stone-crab larvae. Because stone crabs (particularly those in coastal environments) already experience seasonal extremes in pH that are on par with the lower range of expected pCO_2 for the end of the century, we considered using the upper estimate of expected pCO_2 most appropriate for our study. We also tested the hypothesis that those same treatments will result in smaller and morphologically deformed larvae.

2. Materials and Methods

2.1 Stone crab ovigerous female collection

Ovigerous females were collected by Florida Fish and Wildlife using commercial stone crab traps near Pavilion Key (, Florida during the 2014 and 2015 summers (May–August). Females were immediately transported back to the University of Miami's Rosenstiel School's Ocean Acidification Laboratory and were maintained in ambient seawater conditions until larval release. In 2014, larvae that were hatched from 8 different broods were individually raised so that we could measure survivorship and molt-stage duration. In 2015 we mass-reared larvae from which we harvested groups of individuals at certain developmental stages to conduct larval condition (n = 13 broods for stage III, and 8 broods for stage V) and morphology analyses (n = 6 broods for stage III and 7 broods for stage V). Immediately following release, newly hatched larvae were randomly assigned into each of the experimental treatments described below

and larvae from the same brood (i.e., the replicates) were divided among the treatments levels throughout all experiments.

2.2 Experimental design and ocean acidification (OA) system set-up

All experiments consisted of two fully-crossed treatment parameters (i.e., temperature and pCO_2), each with two levels, resulting in a total of four different treatments. The two temperature levels were set at 30°C and 32°C. The lower (control) temperature was based on the mean summer sea surface temperature for the Long Key C-MAN station, in Florida Bay over 1992–2008 years (NOAA National Data Buoy Center, 2016). The upper temperature was based on IPCC (2013) sea-surface temperature projections for the end of the century. The control pCO_2 level was ~450 µatm and corresponded to similar levels at the site of collection (Table 1). The elevated pCO_2 level was set at ~1100 µatm and was based on current IPCC (2013) projections. To achieve the control pCO_2 level, seawater was passed through a sand filter and a 100 µm mesh filter prior to being pumped into the holding reservoirs. Seawater entering the holding reservoir was vigorously aerated until the reservoir was maintained at ~450 µatm. Elevated pCO_2 treatments were achieved by pumping seawater into a separate holding reservoir where pure CO_2 gas was added using venturi injectors and mass flow controllers (MFC; SmartTrak 100, Sierra). Control and elevated-pCO_2 water was then pumped into each of the separate experimental aquaria (7.5 L). Temperature within each experimental aquarium was regulated using heaters and temperature probes, constantly monitored and maintained by AquaControllers (Apex System, Neptune). To avoid shock to the larvae, the use of MFCs and the digitally controlled temperature system allowed us to gradually increase the experimental parameters (~200 µatm and ~0.4 °C per day) to the desired treatment levels over the first 5 days ("ramp-up period") of each experiment.

2.3 Seawater Carbonate Chemistry

To monitor the carbonate chemistry of the OA system, seawater samples were collected from both the holding reservoirs and from each experimental aquaria in 150 mL borosilicate bottles, and were immediately fixed with 100 μL of saturated mercuric chloride. Total alkalinity (A_T) and dissolved inorganic carbon (DIC) were measured at NOAA's Atlantic Oceanographic and Metrological Ocean Acidification Laboratory using Apollo SciTech instruments (AS-ALK2 and AS-C3, respectively) as described by Enochs et al. (2015). Alkalinity and DIC samples were checked for accuracy with certified reference materials (Dickson et al., 2003, Scripps Institution of Oceanography, La Jolla, CA). Carbonate parameters were monitored every other day during the first week of the experiment, and every 5–7 days thereafter. The pH total scale within each experimental aquarium was also measured daily using a handheld pH meter (Oakton) and Ross electrode (Orion 9102BWNP; Thermoscientific), which was calibrated using Tris buffer.

To calculate pCO_2, both A_T and DIC were measured during survivorship and molt-stage duration experiments (2014), while A_T and pH were measured during the larval condition, and morphology experiments (2015). The change in the carbonate parameters between the 2014 and 2015 research season was the result of the DIC analyzer malfunctioning during the 2015 research season. Using A_T, DIC, and pH, allowed the remaining carbonate parameters (DIC, and/or pCO_2) to be determined using CO2SYS software (Robbins et al., 2010). Temperature and salinity of each experimental aquarium were also monitored twice daily throughout all experiments (Orion Ecostar). The carbonate chemistry of seawater samples collected at the site of ovigerous female collection were also analyzed for DIC and TA. Collection of field samples allowed us to model the control pCO_2 levels within the range of the pCO_2 at field collection sites. All field samples were collected between 08:00–12:00 throughout the 2014 (N = 17) and 2015 (N = 10)

experimental season. All control/ambient pCO_2 levels were within ranges reported for other stone crab habitats (Millero et al., 2001; Dufroe, 2012).

2.4 Stone crab larval survivorship and molt-stage duration

Experiments determining the effects of elevated temperature and pCO_2 on larval survivorship and molt-stage duration (MSD) were conducted on larvae reared individually in clear acrylic compartmentalized boxes (80 ml), with the plastic bottoms replaced with nylon mesh (190 µm). Each box was kept in its own water bath to maintain constant experimental temperatures. Larvae (n = 46 per treatment level) from each ovigerous female were placed within each treatment level (i.e., A30, H30, A32, and H32) and were monitored in the boxes to determine the treatment effects on survivorship and MSD. Larvae used during survivorship and MSD experiments came from eight independent broods, and each brood served as a replicate. Ovigerous females were only used once in our experiments. Prior to feeding larvae, *Artemia* were enriched with a lipid diet (Selco, Brine shrimp direct, UT) and fed enriched rotifers. Rotifers that were fed to *Artemia* were also enriched with a high protein lipid diet (One Step, Rotigrow, CA). After enrichment, the *Artemia* were pipetted into each larval chamber (30–40 per individual larval chamber). Larvae were kept on a 14 hr light: 10 hr dark photoperiod that approximated conditions during the time of collection. Survivorship and MSD were monitored by counting exuvia (i.e., molts) and dead larvae at the same time each day. Survivorship was defined as the proportion of individuals that survived from birth to the post-larvae stage, and survival was defined as the chance that an individual will survive to the next stage.

2.5 Larval Condition

Stage I and Stage II larvae never experienced the full experimental treatment conditions (due to gradual ramping up to experimental set points), and therefore, were not used in AFDW analyses. The AFDW experiments used larvae from different broods (i.e., 13 broods for stage III, and 8 broods for stage V), and each brood served as a replicate. A pooled sample, consisting of 50 individuals, was used for stage III larvae, and a pooled sample, consisting of 10 individuals, were used for stage V larvae. The larval dry weight (DW) and ash free dry weight (AFDW) of stage III and V were measured during the 2015 summer using protocols adapted from Nates and McKenney (2000). Larvae were reared in 9L plastic chambers whose sides were composed of nylon mesh to allow for exposure to the treatment conditions. The initial stocking density for each larval rearing chamber was 500 larvae (0.05 larvae per ml). After harvesting, larvae were briefly rinsed, blotted dry on filter paper, and then oven-dried at 60°C for 30 hrs. After being dried, the dry weight per group of larvae was determined using an ultra-microbalance (precision = 0.1 µg; Mettler Toledo UMX2). After measuring dry weight, each sample was combusted (> 450°C) for 12 hrs and reweighed. The AFDW was calculated by subtracting the mass of the ash from the total dry weight.

2.6 Larval morphology

To determine the potential effect of elevated pCO_2 and temperature on larval morphology (n ~ 10) stage III and V larvae were harvested and fixed in 3% glutaraldehyde in 0.1 M phosphate buffer at room temperature (Felgenhauer and Abele, 1983). Stage I and stage II larvae never experienced the full experimental treatment set points, and therefore were not used in morphological analyses in these experiments. After preservation of larvae, a Scanning Electron Microscope (SEM; JEOL JSM-6380LV) was used to take digital images of larvae using methods described by Felgenhauer and Abele (1983). To determine if any differences existed in spination

or size among treatments, larvae were photographed so that the telson spine length (TS), rostrum spine length (RS), dorsal spine length (DS), carapace width (CW), carapace height (CH), whole length (WL), and tail length (TL) could be measured (ImageJ software, Schneider et al., 2012) from digital SEM micrographs (37x, Figure 1). Prior to measurement, digital images of stage III and V larvae were calibrated in ImageJ by determining the number of pixels within the micrometer scale provided by the SEM. The CW was defined as the distance from the base of the rostral spine to the midpoint of the posterior lateral margin of the carapace (Long et al., 2013). The CH was defined as the distance from the base of the dorsal spine to the ventral edge of the carapace (Long et al., 2013). We used larvae from six and seven different broods (replicates) for stage III and stage V larvae respectively.

2.7 Data analysis

The effect of different treatments on survivorship was determined using a failure-time analysis (Cox Proportional Hazard Model), with larval death serving as the 'event', and time since the beginning of the experiment as the 'time until an event occurs'. The Cox regression coefficients (i.e., hazard ratio) were used to estimate the likelihood an individual larva would die under the experimental treatments. Survivorship and MSD experiments were replicated using larvae from eight independent broods (N = 8). To control for variation among broods, larvae from the same female were treated as covariates in the analysis. Comparisons of survivorship among treatments were made using a Log-rank (LR) test.

Stage-specific survival was calculated by dividing the number of larvae surviving at each stage by the initial number of larvae that started each stage. The stage-specific survival did not meet the assumptions of normality and were therefore rank transformed. A repeated measures analysis of

variance (ANOVAR) was then run on the ranked data, with temperature and CO_2 as the main effects, and brood as the within subject factor. The results were Bonferroni corrected to set the alpha level at 0.01, because the stage-specific analysis required five separate tests. Differences among treatments in the molt-stage duration for each larval stage were determined using an ANOVAR with temperature and CO_2 as the main effects, and brood as the within subject factor.

Differences in the mean DW and AFDW for each treatment combination were tested using an ANOVAR with temperature and CO_2 as the main effects, and brood as the within subject factor. Because of the high degree of shared variability among morphological features, principle component analysis (PCA) was used to establish a new set of orthogonal variables that were compared among treatment groups. The contribution of the new variables was determined based on the largest factor loadings for each principle component. The point of inflection on the scree-plot was used to determine the number of PCs to retain. The derived component scores were then analyzed using separate ANOVARs (with brood as a within subject factor) to determine if larval morphology differed among treatments. All statistical analyses were performed using R (R Core Team, 2016).

3. Results

3.1 Seawater Chemistry

After pCO_2 and temperature were gradually increased to the experimental set points, the control's (i.e., ambient temperature and ambient pCO_2; hereafter will be referred to as A30) mean pCO_2 levels were maintained within a narrow range among all treatments (Table 1). Temperature, salinity, and total alkalinity (A_T) also showed little variability after the gradual increase to the

279 experimental set points, for the 2014 and 2015 summer research seasons (Table 1). The pH was
280 lower in the elevated pCO_2 treatments (Table 1).
281
282 ***3.2 Larval survival and development***
283 Survivorship to megalopae was significantly reduced in all treatments (A32, H30, H32) relative
284 to the control (A30, $LR_7 = 272.3$, $P < 0.001$; Fig. 1). There was a 19% absolute decrease in larval
285 survival between the H30 and the control (relative decrease of 37% between treatments; Fig. 1).
286 The Cox regression coefficients (i.e., hazard ratios) were used to express the likelihood an
287 individual would die under the experimental treatments. The hazard ratios indicated that larvae
288 raised in the H30 treatment were 1.5 times more likely to die than larvae raised in ambient
289 conditions (A30). Elevated temperature (A32) resulted in a 36% absolute reduction in survival to
290 megalopae relative to the control, which was almost double the effect of elevated pCO_2 (relative
291 decrease of 71% between treatments; Fig. 1). The combination of both elevated temperature and
292 pCO_2 (H32) resulted in a 41% absolute decrease in individuals surviving to megalopae relative
293 to the control (relative decrease of 80% between treatments; Fig. 1). A comparison of the hazard
294 ratios indicated that mortality was more likely in the A32 and H32 conditions (3.3 and 3.7 times,
295 respectively) than in the control. Pairwise comparisons (log-rank test) indicated that survivorship
296 was significantly lower than the control in all treatments, however, larval survivorship in the
297 A32 and H32 were not significantly different from each other (S1). Female brood (covariate)
298 was observed to have a significant effect on survivorship (Wald $\chi^2 = 45.2$, df = 7, $P < 0.001$).
299
300 Comparisons were also made to determine if there were differences in the stage-specific survival
301 among treatments. The two main effects showed no significant impact on the stage-I survival (S1,

Fig. 2), and there was a significant within-subject (female) effect in stage-I larvae (S1). Stage II larvae had a significantly lower median stage-specific survival in the A32 and H32 treatments (i.e., both elevated temperature treatments were ~5.5% lower than the ambient temperature treatments; S1). Relative to the control, the median stage-specific survival for stage III larvae was also significantly lower by 17% and 31% in the A32 and H32 treatments, respectively (S1, Fig. 2). Stage IV larvae exhibited significant differences in both main effects (S1). Stage IV larvae raised in the H30, A32, and H32 treatments showed decreases in survival of 12%, 31%, and 43%, respectively, when compared to the control. The stage-specific survival of stage-V larvae showed significant differences among the main effects, with the greatest overall decrease in survival compared with the other larval stages (S1, Fig. 2). Relative to the control, the stage-V larvae showed a decrease in survival in the H30, A32, and H32 treatments by 19%, 46%, and 53%, respectively.

Molt-stage durations (MSD) were significantly shorter in the elevated temperature treatments (A32 and H32; S2, Fig. 3). Larvae in the elevated temperature treatment molted ~0.8–1.2 days earlier than larvae raised in the control. There was no effect of elevated pCO_2 on larval MSD until stage V, where development was almost 1 day longer than larvae in the control (0.78 days; S2, Fig. 3). Stage V larvae also had a significant interaction effect among the treatments (S2).

3.3 Larval Condition

The mean DW for stage-III larvae (13 broods used as replicates) showed no significant difference among treatments and on average ranged from 88.5–96.0 µg individual^{-1} (S2). There was no interaction effect between temperature and pCO_2 for stage III DW; however, there was a significant within subject effect (S2). AFDW for stage-III larvae (13 broods used as replicates)

was within a narrow range (56.0–59.0 µg individual^{-1}), and did not differ among treatments (S2). There was no interaction effect between temperature and pCO_2 for stage III AFDW; however, there was a significant within-subject effect (S2). The DW for stage V larvae (8 broods used as replicates) showed no significant difference among treatments and on average ranged from 241–277 µg individual^{-1} (S2). There was no interaction effect between temperature and pCO_2 for stage V DW; however, there was a significant within subject effect (S2). The AFDW (µg individual^{-1}) for stage V larvae showed no significant differences among the main effects (S2) and was also within a narrow range (165–182 µg individual^{-1}). There was no interaction effect, however, there was a significant within-subject effect (S2).

3.4 Larval Morphology

PCA analysis on the morphological measurements of stage III larvae resulted in three principle components (PC's) representing 91.9% of the variation in the data (S3). The PC 1 loadings were negatively associated with all morphometric measurements, and were interpreted as representative of the overall larval size (whole length). The loadings for PC 2 were associated with the dorsal spine, whereas the loadings for PC 3 were interpreted as being the carapace height. PCA analysis on the morphological measurements of stage V larvae resulted in two PC representing 94.7% of the variation (S4). The PC 1 loadings were also negatively associated with all morphometric measurements. The loadings for PC 2 were associated with the dorsal spine and was interpreted as representing overall animal size (height). The derived component scores were compared among the main effects using an ANOVAR for both stage III and V larvae, and showed no significant differences for larval morphology (S4); there was however significant brood effects (S3 and S4).

4. Discussion

Our results demonstrate that the survivorship and development of stone crab larvae were sensitive to elevated temperature and pCO_2. The detrimental effect of elevated temperature, however, was more than two times greater than elevated pCO_2. The stone crabs sensitivity to acidified conditions was intriguing since species that typically live in habitats that experience variability in pH conditions (i.e., coastal areas after runoff events) might be at an advantage for adaptive responses to ocean acidification (Hofmann et al. 2010). For instance, some crustacean species such as the Tanner crab (*Chionoecetes bairdi*) also live in variable pH habitats, yet acidified conditions appear to have no substantial effect on larval survivorship (Long et al. 2016). During our study, field temperature ranged from 28.2–31.3°C and pCO_2 ranged from 392–596 µatm (pH range 7.95–8.18) at the ovigerous crab collection site. Despite this natural variability, larval mortality still increased during exposure to both elevated temperature and pCO_2 treatments; however in combination they did not impact larval condition or morphology throughout development, which could indeed reflect some degree of tolerance.

4.1 Larval survival

The elevated pCO_2 (H30) treatment showed a decrease in survivorship resulting in individuals being 1.5 times more likely to experience mortality than the control, however, elevated temperature more than doubled the likelihood that an individual would die. The impact of elevated temperature showed the greatest impact on stone crab larval survivorship (regardless of pCO_2), causing increases in mortality that were 3.3 (A32) and 3.7 (H32) times greater than the control (A30). Similar negative effects of elevated pCO_2 have been reported for other crab species including juveniles of the red king crab, *Paralithodes camtschaticus,* and the Tanner crab, *Chionoecetes bairdi* (Long et al., 2013a), while elevated pCO_2 and temperature negatively

374 impacted larvae of the spider crab, *Hyas araneus* (Walther et al., 2010). Larvae in our study only
375 experienced a 2 °C increase in temperature; however, the significantly lower survivorship we
376 observed agrees with previous stone crab work that reported higher larval mortality when
377 temperatures reach 35 °C (Brown et al., 1992).
378
379 Elevated temperature has long been cited as one of the most critical environmental factors that
380 directly impacts crustacean metabolic rates, molt-stage duration, and development time (Costlow
381 et al., 1960; Costlow and Bookhout, 1971). Although the physiological mechanisms contributing
382 to the decrease in survival were not examined in this study, elevated temperature is known to
383 impact metabolic activity, growth, circulation, and ventilator mechanisms among the different
384 life stages of crustaceans (Frederich and , 2000; Storch et al., 2011). Once an individual
385 reaches its temperature threshold the organism moves into anaerobic metabolism which limits
386 oxygen supply at the cellular level (et al., 2005; Storch et al., 2011). Additionally,
387 elevated temperatures are known to increase metabolism (Leffler, 1972; Arnberg et al., 2013).
388 For example, the northern shrimp *Pandalus borealis*, showed a metabolic increase of ~20% when
389 exposed to both higher temperatures and pCO_2 conditions (Arnberg et al., 2013). The stress
390 associated with molting in crustaceans can further add to metabolic demands, because molting is
391 often accompanied by a large increase in oxygen consumption, resulting in a 2-fold increase in
392 metabolism (Roberts, 1957; Leffler, 1972). High mortality at elevated temperatures could also be
393 the result of larvae experiencing heat stress, which is suggested to disrupt enzymatic and
394 hormonal systems that regulate the molt cycle (Anger, 1987). The stability and function of certain
395 enzymes and proteins may not function at elevated temperatures or elevated pCO_2, resulting in
396 some pathways either not operating or working less efficiently (Somero, 1995; Hofmann and
397 Todgham, 2010).

398

399 *4.2 Molt-stage duration*

400 Development across all larval stages was predominately temperature dependent, which was
401 indicated by a 13% and 14% shorter molt-stage duration in the H32 and A32 levels, respectively.
402 A shorter molt-stage duration was expected, as higher temperature is known to accelerate molting
403 in both larval and juvenile coastal and estuarine crustacean species like *Callinectes sapidus*
404 (Leffler, 1972), *Cancer irroratus* (Johns, 1981), and *Cancer magister* (Kondzela and Shirley,
405 1993). Coastal and estuarine crustaceans (i.e., *Sesarma, Callinectes, Menippe* spp.) exposed to
406 elevated temperatures will experience an increase in metabolic processes, resulting in larvae
407 progressing through each stage more quickly (Costlow et al., 1960; Ong and Costlow, 1970;
408 Leffler, 1972). For example, increased seawater temperature will accelerate growth, until a
409 threshold is reached, beyond which growth declines. However, rapid growth is also associated
410 with physiological costs, such as depletion of energy reserves that may be required in later stages
411 (Kurihara et al., 2008).

412

413 The present study showed that exposure to elevated pCO_2 also resulted in a significantly longer
414 (~12%) molt-stage duration in stage-V larvae, therefore prolonging the transition into the post-
415 larval stage. Slower development under elevated seawater pCO_2 has been previously reported for
416 the larvae of the spider crab, *H. araneus* (Walther et al., 2010), and for the shrimp *Palemon.*
417 *pacificus* (Kurihara et al., 2008). However, both of these studies observed significant delays in
418 development only when CO_2 levels were well above projections for the end of the next century (~
419 2000 μatm in Kurihara et al., 2008; 3000 μatm in Walther et al., 2010). The slight delay (~1 day)
420 observed in the present study could increase the susceptibility of late-stage stone-crab larvae to
421 planktotrophic predators. The lack of a significant delay in development, which lasts for several

422 days or weeks under elevated pCO_2, suggests that pCO_2 conditions forecast for 2100 will likely
423 not have any significant biological impacts on stone crab larval development.
424
425 *4.3 Larval weight*
426 Our results for the larval ash free dry weight (AFDW) do not support the hypothesis that larval
427 condition was impacted by elevated pCO_2 or elevated temperatures. We expected larval condition
428 (AFDW) would be lower in acidified conditions; however this was not the case. This result was
429 unexpected, and the reason for the indifference in AFDW is unknown, but could be related to
430 conducting experiments during different years and from using larvae from different broods than
431 in 2014 survivorship experiments. The observed within subject effects suggests significant
432 variability among parents, and indicates that some broods were more tolerant to elevated pCO_2
433 and temperature than other broods. The brood-specific responses observed here are likely a
434 consequence of variability among females (e.g., prior exposure to low pH conditions or genetic
435 variation among broods) which could allow the species to be resilient to future ocean changes
436 (Ceballos-Osuna et al., 2013; Carter et al., 2013). Previous work that quantified larval condition
437 under elevated pCO_2 and temperature scenarios for other Brachyuran crabs report similar patterns
438 in both larval condition and survival as reported here. For instance, larval survivorship decreased
439 in *H. araneus,* but larval lipid ratios showed no change under elevated pCO_2 (380–3000 ppm) and
440 elevated temperature (Walther et al., 2010). Additionally, the Tanner crab, *C. bairdi* also
441 exhibited no significant change in larval-condition index, yet, juveniles elicited a 130% increase
442 in mortality at elevated pCO_2 (~800 µatm, pH = 7.8; Long et al., 2013b). Typically, reductions in
443 larval condition and survivorship are associated with elevated pCO_2 and elevated temperature,
444 which affect metabolic processes that interfere with the function of certain pH-dependent
445 enzymes or hormones necessary for molting. The CO_2 diffuses into the larval body to acidify the

haemolymph (Pörtner et al., 2004). Such changes were hypothesized to occur in post-larvae of *H. araneus* that were exposed to OA and elevated temperatures, however, the AFDW results reported show no differences between treatments.

4.4 Larval morphology

The morphology of stone crab larvae was also not affected by elevated pCO_2 and temperature. This result is in contrast to other crustacean studies, which show that the larval morphology of red king crab *P. camtschaticus* (Long et al., 2013b) were 4% larger under acidified conditions. Our results suggest that the morphology of stone crab larvae will not be impacted by future changes in seawater pCO_2 or temperature. However, there is potential for elevated pCO_2 and temperature to impact the size, shape, and shell thickness, and hardness of post-larval and juvenile stages of stone crabs, given that some crustaceans incorporate greater amounts of calcium into the exoskeleton of late-life stages (Richards, 1958; Arnold et al., 2009; Walther et al., 2011; Coffey et al., 2017). The lack of any differences in larval skeletal content among treatments is likely attributed to the molting process in larval crustaceans. During molting, crustacean larvae inflate their body with the surrounding seawater, which permits Ca^{2+} ions to permeate via diffusion across the thin exoskeleton of the larvae (Anger, 2001; Walther et al., 2011). Once larvae molt, and develop into post-larvae stages, a greater amount of Ca^{2+} is embedded into the carapace with each progressive molt. The highest Ca^{2+} content is usually found in the oldest post-larvae stages and in juveniles (Arnold et al., 2009; Walther et al., 2011). Calcification has also been shown to increase with higher salinities in some crustaceans (Egilsdottir et al., 2009) regardless of pCO_2 level; however, salinity in our experiments was similar at 35–37 across treatments.

Elevated seawater temperatures appear to have a greater impact on stone crabs than the effects of elevated pCO_2, suggesting that some components of larval development may be tolerant to future changes in carbonate chemistry. The significant decline in survivorship observed at elevated seawater temperatures is especially concerning considering that seawater temperatures are predicted to increase at a faster rate than increases in pCO_2 (IPCC, 2013). Historical trends already indicate that the rate of sea-surface warming, projected for the 21st century, is five times faster than the 0.6°C warming rate documented in the 20th century (Kerr, 2004). Additionally, some stone crab habitats, for example the Florida Keys, have experienced a 0.8 °C increase in sea surface temperature over the last century (Kuffner et al., 2012). Such conditions are potentially problematic for stone crabs since they are a subtropical species and already live close to their thermal limit, especially during the summer reproductive season. For instance, over the last few years some stone crab habitats in the Florida Keys have already experienced episodic increases in temperature (≥ 32°C; National Data Buoy Center, 2016) which could be contributing significantly to larval mortality. The continued increase in seawater temperatures projected for 2100 may serve as a potential bottleneck for the population by reducing the number of larvae that survive. The susceptibility of stone crab larvae to elevated temperatures could therefore promote a northward range expansion as ocean temperatures continue to increase. Elevated seawater temperatures, however, are likely to cause a decline in the stone crab larval population in the absence of phenotypic or evolutionary adaptation (Long et al., 2013) and could threaten the future sustainability of the fishery.

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References

Anger, K., 1983. Moult cycle and morphogenesis in *Hyas araneus* larvae (Decapoda, Majidae),

–302

517 Anger, K., 1987. Energetics of spider crab *Hyas araneus* megalopa in relation to temperature and
518 the moult cycle. Mar. Ecol. Prog. Ser. 36:115–122.
519
520 2001. The biology of decapod crustacean larvae. Crustac Issues 14, Balkema
521 Publishers, Lisse.
522
523 Arnberg, M., Calosi, P., Spicer, J.L., Tandberg, A.H.S., Nilsen, M., Westerlund, S., Bechmann,
524 R.K., 2013. Elevated temperature elicits greater effects than decreased pH on the development,
525 feeding and metabolism of northern shrimp (*Pandalus borealis*) larvae. Mar. Biol. 160: 2037–
526 2048.
527
528 Arnold, K.E., Findlay, H.S., Spicer, J.I., Daniels, C.L., Broothroyd, D., 2009. Effect of CO_2-
529 related acidification on aspects of the larval development of the European lobster, *Homarus*
530 *gammarus* (L.). Biogeosciences 6:1747–1754.
531
532 Bauer, J.E., Cai, W., Raymond, P.A., Bianchi, T.S., Hopkinson, C.S., Regnier, P.A.G., 2013. The
533 changing carbon cycle of the coastal ocean. Nature 504:61–70.
534
535
536
537 –
538

Brown, S.D., Bert, T.M., Tweedale, W.A., Torres, J.J., Lindberg, W.J., 1992. The effects of temperature and salinity on survival and development of early life stage Florida stone crabs *Menippe mercenaria* (Say). J. Exp. Mar. Biol. Ecol. 157:115–136.

Byrne, M., 2011. Impact of ocean warming and ocean acidification on marine invertebrate life history stages: vulnerabilities and potential for persistence in a changing ocean. Oceanogr. Mar. Biol. Annu. Rev. 49:1–42.

Calderia, K., Wickett, M.E., 2003. Anthropogenic carbon and ocean pH. Nature 425:365.

Carter, H.A., Ceballos-Osuna, L., Miller, N.A., Stillman, J.H., 2013. Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. J. Exp. Biol. 216:1412–1422.

Ceballos-Osuna, L., Carter, H.A., Miller, N.A., Stillman, J.H., 2013. Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes*. J. Exp. Biol. 216:1405–1411.

Conover, W.J., Iman R.L., 1981. Rank Transformations as a bridge between parametric and nonparametric statistics. Am. Stat. 35(3):124–129.

Costlow, J.D. Jr., Bookhout, C.G., Monroe, R., 1960. The effect of salinity and temperature on larval development of *Sesarma cinereum* (Bosc). Biol. Bull. 118:183–202.

Costlow, J.D. Jr., Bookhout, C.G., 1971. The effect of cyclic temperatures on larval development in the mud-crab *Rhithropanopeus harrisii*. 4th European Marine Biology Symposium. Cambridge University Press, Cambridge.

Dickson, A.G., Afghan, J.D., Anderson, G.C., 2003. Reference materials for oceanic CO_2 analysis: A method for the certification of total alkalinity. Mar. Chem. 80:185–197.

Dufroe, C.M., 2012. Spatial and temporal variations in the air-sea carbon dioxide fluxes of Florida Bay. Ph.D. dissertation, University of South Florida, Tampa, FL, unpublished.

–

Engle, V., 1958. The blue crab and its fishery in Chesapeake Bay. Part 1 – Reproduction, early larval development, growth, and migration. Commercial Fisheries Rev. 20:6–17.

–

587 Ekstrom, J.A., Suatoni, L., Cooley, S.R., Pendleton, L.H., Waldbusser, G.G., Cinner, J.E., Ritter,
588 J., Langdon, C., Van Hooidonk, R., Gledhill, D., Wellman, K., 2015. Vulnerability and
589 adaptation of US shellfisheries to ocean acidification. Nat. Clim. Change. 5:207–214.
590
591
592 –
593
594 Felgenhauer, B.E., Abele, L.G., 1983. Ultrastructure and functional morphology of feeding and
595 associated appendages in the tropical fresh-water shrimp *Atya innocuous* (Herbst) with notes on
596 its ecology. J. Crustac. Biol. 3: 336–363.
597
598 Florida Fish and Wildlife Conservation Commission, 1998-2016. Commercial fisheries landings
599 summaries. https://public.myfwc.com/FWRI/PFDM/ReportCreator.aspx; accessed January 4,
600 2017.
601
602 Frederich, M., , H.O., 2000. Oxygen limitation of thermal tolerance defined by cardiac
603 and ventilatory performance in spider crab, *Maja squinado*. Am. J. Physiol. Regul. Integr. Comp.
604 Physiol. 279:R1531–R1538
605
606 Gandy, R.L., Crawford, C., Bert, T.M., Ewing, L., Black, T., 2010. Stone Crab Fishery-
607 Independent Monitoring Program Annual Report. Florida Fish and Wildlife Conservation
608 Commission, Fish and Wildlife Research Institute (FWC/FWRI) Publication Number IHR 2010-
609 004 FWC/FWRI, St. Petersburg, Florida, USA. 16 pp.
610

Gazeau, F., Quiblier, C., Jansen, J.M., Gattuso, J.P., Middleburg, J.J., Heip, C.H.R., 2007. Impact of elevated CO_2 on shellfish calcification. Geophys. Res. Lett. 34:1–5.

J. Exp. Mar. Biol. Ecol. 500:140–146. https://doi.org/10.1016/j.jembe.2017.09.001

tal

–

–

Hilmi, N., Allemand, D., Dupont, S., Safa, A., Haraldsson, G., Nunes, P.A., Moore, C., Hattam, C., Reynaud, S., Hall-Spencer, J.M., Fine, M., 2013. Towards improved socio-economic assessments of ocean acidification's impacts. Mar. Biol. 160:1773–1787.

Hofmann, G.E., Todgham, A.E., 2010. Living in the now: physiological mechanisms to tolerate a rapidly changing environment. Annu. Rev. Physiol. 72:127–45.

organism-to-ecosystem perspective. Annu. Rev. Ecol. Evol. Syst. 41:127–147.

Intergovernmental Panel on Climate Change., 2013. The physical science basis. Working Group I contribution to the 5th assessment report of the Intergovernmental Panel on Climate Change. Cambridge University Press.

Kerr, R., 2004. Three degrees of consensus. Science 305:932–934.

Krimsky, L.S., Epifanio, C.E., 2008. Multiple cues from multiple habitats: effects on metamorphosis of the Florida stone crab, *Menippe mercenaria*. J. Exp. Mar. Biol. Ecol. 358:178–184.

Krimsky, L.S., Gravinese, P.M., Tankersley, R.A., Epifanio, C.E., 2009. Patterns of larval release in the Florida stone crab, *Menippe mercenaria*. J. Exp. Mar. Biol. Ecol. 373:96–101.

Kurihara, H., Matsui, M., Furukawa, H., Hayashi, M., Ishimatsu, A., 2008. Long-term effects of predicted future seawater CO_2 conditions on the survival and growth of the marine shrimp *Palemon pacificus*. J. Exp. Mar. Biol. Ecol. 367:41–46.

Kroeker, K.J., Kordas, R.L., Crim, R., Hendriks, I.E., Ramajo, L., Singh, G.G., Duarte, C.M., Gattuso, J.P., 2013. Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Glob. Change Biol. 19(6): 1884–1896.

Leffler, C.W., 1972. Some effects of temperature on the growth and metabolic rate of juvenile blue crabs, *Callinectes sapidus*, in the laboratory. Mar. Biol. 14:104–110.

Lindberg, W.J., Marshall, M.J., 1984. Species profiles: life histories and environmental requirements of coastal fishes and invertebrates (south Florida)—stone crab. U.S. Fish Wildlife Service. FWS/OBS-82/11.21. U.S. Army Corps of Engineers. TR EL-82-4. 17 pp.

Long, W.C., Swiney, K.M., Foy, R.J., 2013b. Effects of ocean acidification on the embryos and larvae of red king crab, *Paralithodes camtschaticus*. Mar. Pollut. Bull. 69:38–47.

Long, W.C., Swiney, K.M. Foy, R.J., 2016. Effects of high pCO_2 on Tanner crab reproduction

Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., H.O., 2009. Physiological basis for high CO_2 tolerance in marine extothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331.

–

Millero, F.J., Hiscock, W.T., Huang, F., Roche, M., Zhang, J.Z., 2001. Seasonal variation of the carbonate system in Florida Bay. Bull. Mar. Sci. 68:101–123.

Mohamedeen, H., Hartnoll, R.G., 1989. Larval and postlarval growth of individually reared specimens of the common shore crab *Carcinus maenas* (L.). J. Exp. Mar. Biol. Ecol. 134:1–24.

–

705 Morgan, S.G., 1989. Adaptive significance of spination in estuarine crab zoeae. Ecology 70: 464–
706
707
708 Nates, S.F., McKenney, C.L., 2000. Growth and variations in lipid classes and fatty acid
709 composition during larval development of the stone crab, *Menippe adina* Williams and Felder,
710 1986. Invertebr. Reprod. Dev. 37:157–165.
711
712 Naylor, E. 1965. Effects of heated effluents on marine and estuarine organisms. Adv. Mar. Biol.
713 3: 63–102.
714
715 NOAA National Data Buoy Center, 2016.
716 http://www.ndbc.noaa.gov/view_climplot.php?station=lonf1&meas=st (accessed 3 Feb 2016).
717
718 Ong, K., Costlow, J.D., 1970. The effect of salinity and temperature on the larval development of
719 the stone crab, *Menippe mercenaria* (Say), reared in the laboratory. Chesap. Sci. 11:16–29.
720
721
722
723 –
724
725 Pörtner, H.O., 2008. Ecosystem effects of ocean acidification in times of ocean warming: a
726 physiologist's view. Mar. Ecol. Prog. Ser. 373:203–217.
727
728 –

729

730 Pörtner, H.O., Langenbuch, M., Reipschläger. A., 2004. Biological impact of elevated ocean CO_2

731 –

732

733 Pörtner, H.O., Langenbuch, M., Michaelidis, B., 2005. Synergistic effects of temperature

734 extremes, hypoxia, and increases in CO_2 on marine animals: from Earth history to global change.

735 J. Geophys. Res. 110: C09S10.

736

737 Pörtner, H.O., Lucassen, M., Storch, D., 2005b. Metabolic biochemistry: its role in thermal

738 tolerance and in the capacities of physiological and ecological function. In: Farrell A, Steffensen

739 JF (eds) The physiology of polar fishes. Elsevier, Amsterdam, p 79–154

740

741 Pörtner, H.O., Bennett, A.F., Bozinovic, F., Clarke, A., Lardies, M.A., Lucassen, M., Pelster, B.,

742 Schiemer, F., Stillman, J.H., 2006. Trade-offs in thermal adaptation: The need for a molecular to

743 ecological integration. Physio. Biochem. Zool. 79:295–313.

744

745

746 –

747

748 R Development Core Team., 2016. R: A language and environment for statistical computing. R

749 Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL

750 http://www.R-project.org.

751

Rastrick, S.P.S., Calosi, P., Calder-Potts, R., Foggo, A., Nightingale, G., Widdicombe, S., Spicer, Living in warmer, more acidic oceans retards physiological recovery from tidal emersion in the velvet swimming crab, *Necora puber*.

Robbins, L.L., Hansen, M.E., Kleypas, J.A., Meylan, S.C., 2010. CO2calc—A user-friendly seawater carbon calculator for Windows, Max OS X, and iOS (iPhone): U.S. Geological Survey Open-File Report 2010; 1280, 17 p.

774 Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image
775 analysis. Nat. Methods 9:671–675.
776
777 Somero, G.N., 1995. Proteins and temperature. Annu. Rev. Physiol. 57:43–68.
778
779 Storch, D., Fernadez, M., Navarrete, S.A., Pörtner, H.O., 2011. Thermal tolerance of larval stages
780 of the Chilean kelp crab *Taliepus dentatus*. Mar. Ecol. Prog. Ser. 429:157–167.
781
782 Swingle, J.S., Daly, B., Heck, J., 2013. Temperature effects on larval survival, larval period, and
783 health of hatchery-reared red king crab, *Paralithodes camtschaticus*. Aquaculture 384:13–18.
784
785 Tomanek, L., 2010. Variation in the heat-shock responses and its implications for predicting the
786 effect of global climate change on species' biogeographical distribution ranges and metabolic
787 costs. J. Exp. Biol. 213:971–979.
788
789
790
791
792
793 –
794
795 Walther, K., Anger, K., Pörtner, H.O., 2010. Effects of ocean acidification and warming on the
796 larval development of the spider crab *Hyas araneus f*rom different latitudes (54°vs. 79°N). Mar.
797 Ecol. Prog. Ser. 417:159–170.

798

799 Walther, K., Sartoris, F.J., Pörtner, H.O., 2011. Impacts of temperature and acidification on larval
800 calcium incorporation of the spider crab *Hyas araneus* from different latitudes (54 vs. 79N). Mar.
801 Biol. 158:2043–2053.

802

803 Whiteley, N.M., 2011. Physiological and ecological responses of crustaceans to ocean
804 acidification. Mar. Ecol. Prog. Ser. 430:257–271.

805

806 Widdicombe, S., Spicer, J.I., 2008. Predicting the impact of ocean acidification on benthic
807 biodiversity: what can animal physiology tell us? J. Exp. Mar. Biol. Ecol. 366:187–197.

808

Figure Captions

Figure 1: Scanning electron microscope image of a stage V larva depicting the morphometric variables used in this study. Morphometric measurements included the telson spine (TS), dorsal spine (DS), rostrum spine (RS), carapace height (CH), carapace width (CW), tail length (TL), and whole length (WL). Image was taken at 37x, 5kV, and 30 SEI by Philip Gravinese.

Figure 2. Cumulative survivorship of *M. mercenaria* larvae throughout larval development during exposure to different combinations of pCO_2 and temperature. The 95% confidence intervals are indicated by the shaded regions. Larvae from eight different broods were used in the analyses (i.e., N = 8 replicates). Curves with different letters are significantly different at $\alpha = 0.05$. A30 (i.e., the control) represents the ambient pCO_2 and ambient temperature treatment. H30 is the elevated pCO_2 and ambient temperature treatment. A32 is the ambient pCO_2 and elevated temperature treatment, and H32 is the elevated pCO_2 and elevated temperature treatment.

Figure 3. Box and whiskers plot of stage-specific survivorship for *M. mercenaria* larvae during exposure to different combinations of pCO_2 and temperature. Larvae from eight different broods were used in the analyses (i.e., N = 8 replicates). Boxes with similar letters are not significantly different from each other (ANOVAR). Control (white) is ambient CO_2 and temperature, H30 (blue) is the elevated pCO_2 and ambient temperature treatment. A32 (light red) is the ambient pCO_2 and elevated temperature treatment, and H32 (dark red) is the elevated pCO_2 and elevated temperature treatment.

Figure 4. Mean (days ±SE) molt stage duration of *M. mercenaria* larvae throughout larval development during exposure to different combinations of pCO_2 and temperature. Larvae from

eight different broods were used in the analyses (i.e., N = 8 replicates). Letters above the bars represent differences between the treatments at $\alpha = 0.05$.

Figure 1

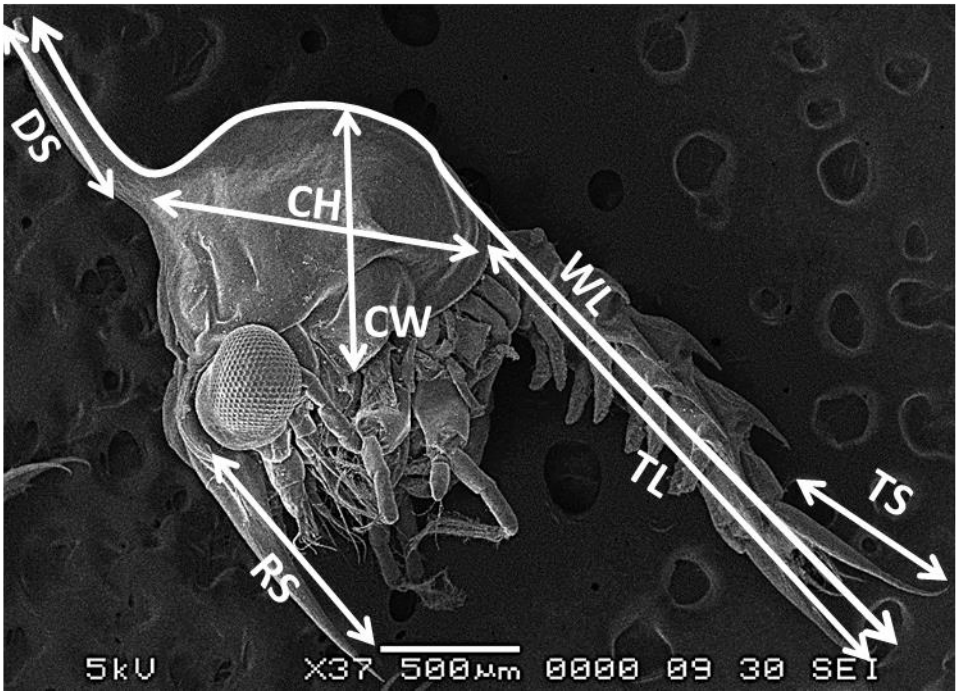
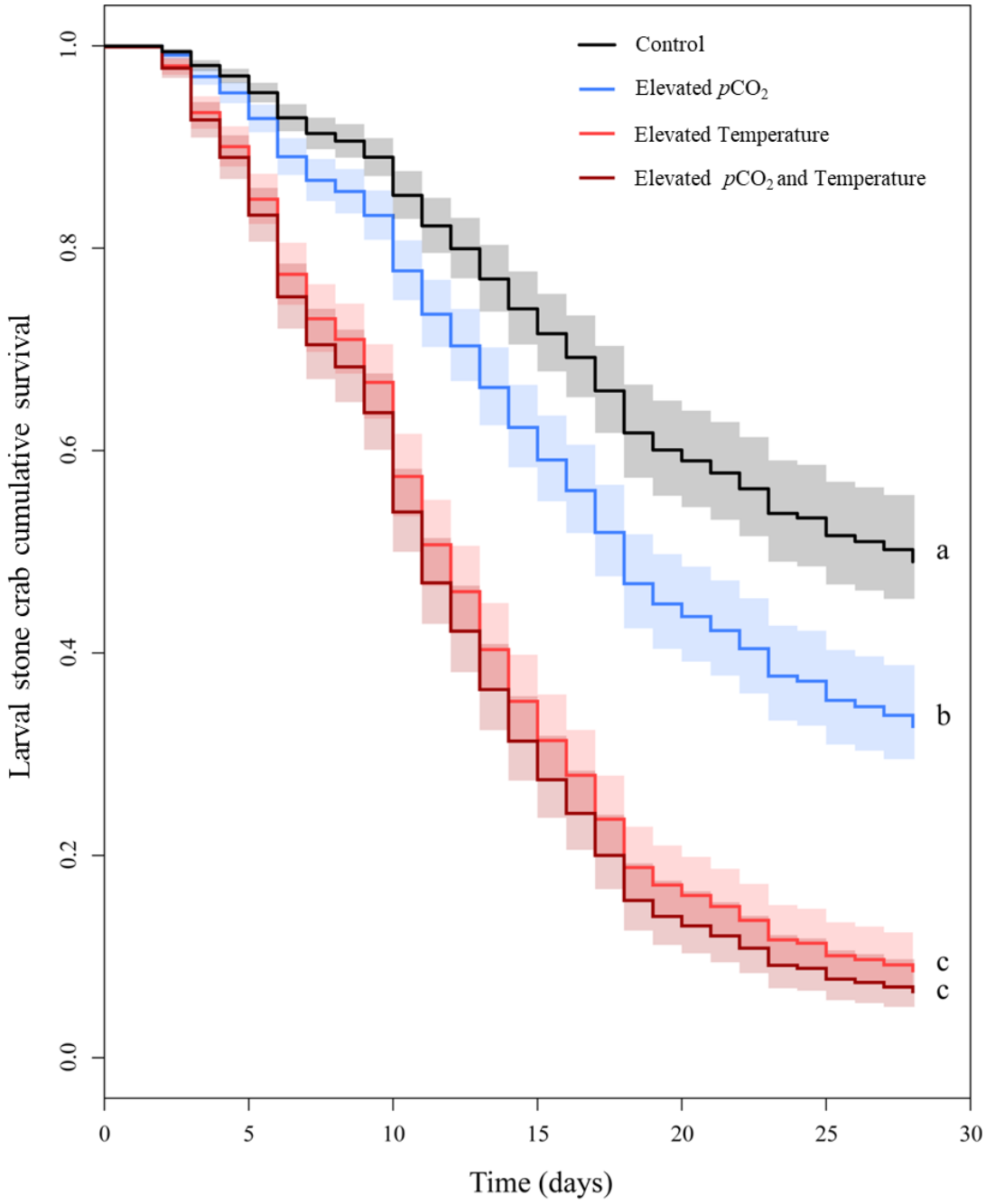


Figure 2


Control
Elevated pCO_2
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Elevated pCO_2 and Temperature
Larval stone crab cumulative survival
0.0
0.2
0.4
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Time (days)
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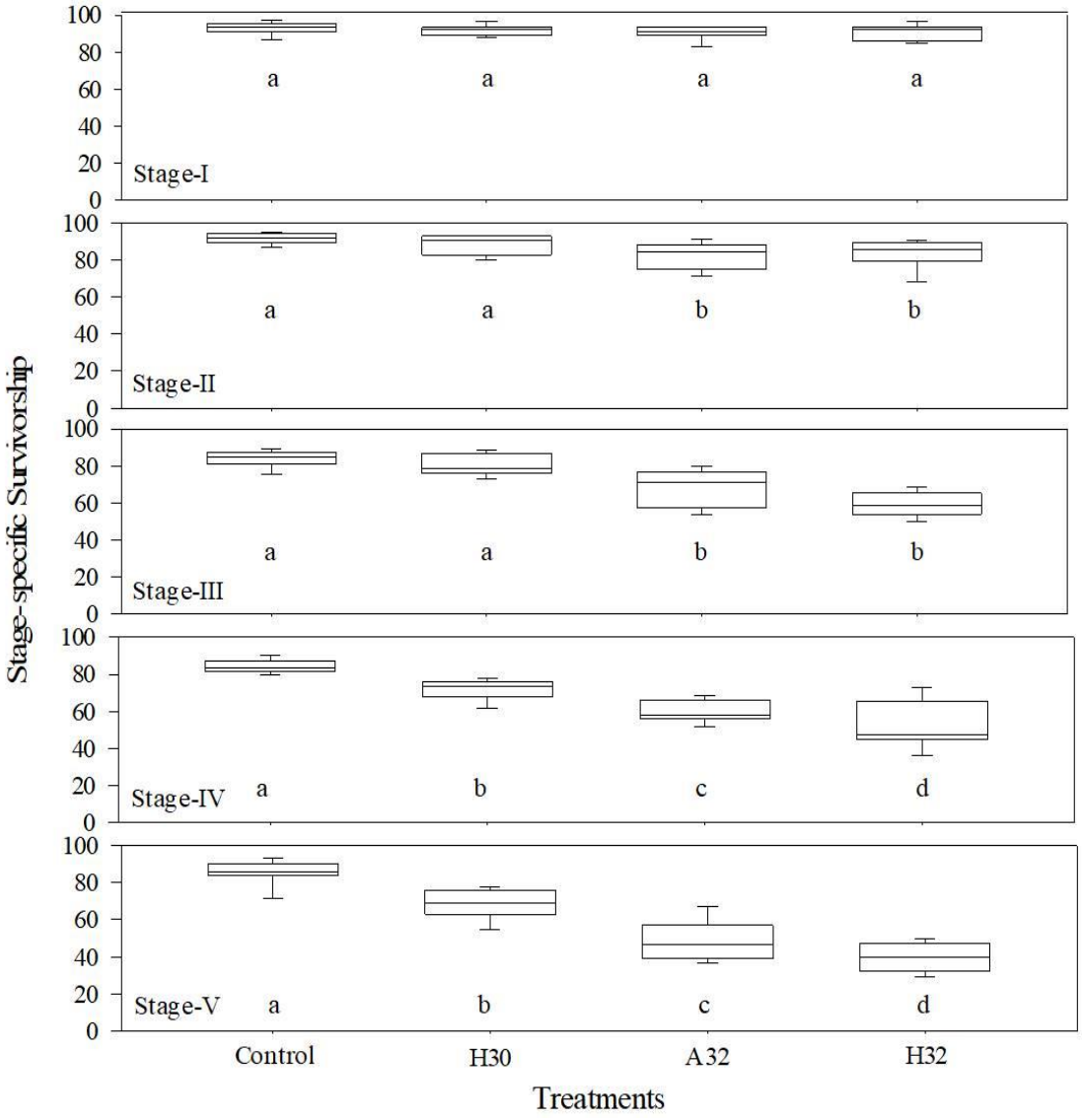
c

877 Figure 3



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885 Figure 4

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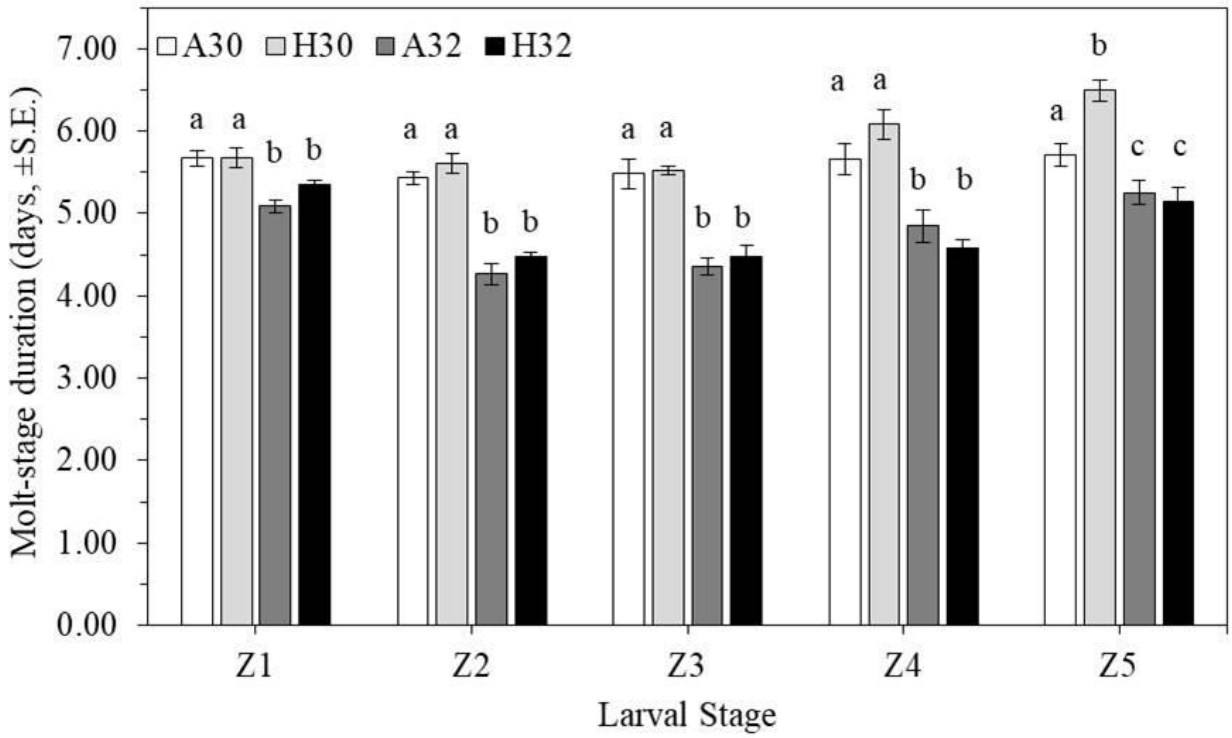

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