

OCEAN ACIDIFICATION EFFECTS ON STATOLITH STRUCTURE, ORIENTATION AND CHEMOSENSORY ABILITY, AND EARLY LIFE HISTORY STAGES OF SEVERAL SPECIES OF MARINE SHRIMP

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Abstract. Acidification of ocean waters has been shown to affect numerous biological functions across many taxa. Among those most affected are calcifying organisms. Marine shrimp that incorporate calcium carbonate sediment into their statoliths within their statocysts are believed to be negatively impacted by decreases in seawater pH. Statocysts are organelles used for balance and orientation in response to gravity and movement. It was predicted that the deterioration of statoliths would greatly affect the shrimp's ability to respond to gravity and orient themselves properly. This was tested by comparing self-righting times between unilaterally, bilaterally, and sham statolith-ablated *Litopenaeus stylirostris*. The degradation of statoliths due to ocean acidification's deleterious effects on internal physiology and the subsequent change in orienting behavior was examined within this study by testing *Caridea* shrimp's ability to use chemosensory cues to detect and navigate through a maze to find food source, and subsequently dissecting out statoliths to determine size and degree of degradation between those housed in acidified seawater ($\text{pH} = 7.77 \pm 0.01$) and those in ambient seawater ($\text{pH} = 8.16 \pm 0.03$). The effects of acidified seawater on survivorship at different life history stages was also determined in *Synalpheus carinatus* eggs, *Caridea* larvae, and *Stenopodidea* larvae in order to help fully understand the impacts of ocean acidification on fitness and survivorship on marine shrimp. Findings show that statoliths are indeed a critical part of basic survival behavior. Bilaterally statolith-ablated *Litopenaeus stylirostris* took significantly longer to self-right themselves when turned upside down. *Caridea* shrimp housed in acidified seawater took significantly longer to orientate themselves within the maze and find the food than those housed in ambient seawater. This is believed to be because statolith size decreased in those housed in acidified water for up to 22 days. *Synalpheus carinatus* eggs, *Caridea* larvae, and *Stenopodidea* larvae, all had a lower survival rate in the acidified seawater treatment compared to those in ambient seawater. These results suggest that acidified seawater with a pH of 7.77 ± 0.01 , consistent with predicted sea surface pH models by the year 2100, is deleterious for the shrimp, and possibly other crustaceans that possess calcium carbonate based statoliths.

Key words: ocean acidification; shrimp; statoliths; survivorship; Moorea; French Polynesia, larvae

INTRODUCTION

Increase in anthropogenic carbon dioxide emissions during the past century has led to many global climatic and environmental changes. With increases in CO_2 in the atmosphere comes increases of CO_2 gas exchange with the ocean surface waters.

Approximately one-third of the anthropogenic CO_2 produced in the past 200 years has been taken up by the oceans (Sabine et al. 2004). The increases in partial pressure of CO_2 in the water greatly alters sea water chemistry, decreasing the pH of the water making it more acidic (Hoegh-Guldberg et al. 2007). The altered pH of ocean water has been shown to greatly

affect a wide range of marine organisms in all zones of the ocean (Fabry 2008), specifically those that precipitate carbonate structures from the water column (Hofmann et al. 2010). This increase in acidity not only makes it more difficult for organisms to calcify their shells or other parts of their bodies, but also degrades existing calcified objects.

Ocean acidification poses challenges for the diverse groups of organisms that form calcified shells, skeletons, and internal structures such as otoliths and statoliths (Hofmann et al. 2010). Among these organisms, corals have been the most extensively studied (Gattuso et al. 1998; Kleypas et al. 1999, Langdon et al. 2000). External calcifiers such as bivalves (Michaelidis et al. 2005), and gastropods (Shirayama and Thornton 2005), have also been studied in relation to ocean acidification. In contrast, little work has focused on organisms that contain internal carbonate structures and how they are affected by acidic sea water.

Calcification is linked intimately to other physiological processes that are likely to be affected by ocean acidification (Hofmann et al. 2010). Increased CO₂ levels have been shown to impair fish olfactory systems (Munday et al. 2009), jellyfish reproduction (Winans and Purcell 2010), and metabolic processes of many organisms, including decapod crustaceans (Kell and Hoffman 2012). Mineralization of the various types of gravity receptors, such as statocysts, are also hypothesized to be affected by the changing carbonate chemistry of seawater, but little is known about how it might impact overall fitness of the organism. Potential impacts likely depend on the ability of the organisms to regulate the acid-base balance in the tissues surrounding those structures. (Fabry et al. 2008).

Statocysts are important balance organs in a number of invertebrates, including many gelatinous plankton, crustacean zooplankton, and benthic invertebrates (Winans and Purcell 2010). Statocysts are balance sensory receptors composed of a sac-like structure containing a statolith within, that may either be biologically mineralized or incorporated from surrounding sediment. Statocysts are also

lined with numerous innervated setae that detect movement of the statolith in response to changes in orientation as the organism moves. The composition of statoliths varies among taxa, but many are composed of calcium carbonate (Fabry et al. 2008). Potential effects of ocean acidification on biomineralization of statoliths are not understood (Hofmann et al. 2010). The consequences of statolith dissolution, however, could include an inability of various taxa to orient to gravity as well as to induce behavioral abnormalities (Haywood & Alexander, 1982).

Crustaceans are an ideal group to examine to uncover the effects of acidified sea water on statoliths. They have been extensively studied in terms of behavior and distribution (Cohen 1955, Xinzhen LI 2008, Ries et al. 2009). Shrimp in particular are useful model organisms for studies on the effects of ocean acidification on statolith morphology and orientation ability (Kurihara et al. 2008). They are abundant and easy to manipulate in the lab. Their morphology also allows for easy access to statocysts without greatly damaging the organism (Niel 1975). Many crustaceans are also non-selective detritivores that scavenge for food, this makes it easy to maintain them and find a suitable food source to elicit food-seeking behavior (Costero and Meyers 1993). Their chemoreceptive ability to detect and locate food has been extensively studied (Carr 1978, Zimmer-Faust 1989). The life cycle of all genera of shrimp has also been well characterized, allowing for suitable comparison of experimental larval survivability to expected survivability (Byrne 2011, Bechmann et al. 2011, Kurihara 2008). Early life history stages are the most energetically demanding stages for marine crustaceans (Bechmann et al. 2011) and the point at which organisms are the most vulnerable to environmental change (Kurihara 2008). Future changes in ocean acidity will impact population size and dynamics and community structure of these marine organisms, so it is crucial to understand not only how ocean acidification affects adults but also eggs and larvae.

The overarching goal of this study was to determine how ocean acidification affects

shrimp survivability and overall fitness. This study sought to determine (1) the functional importance of statoliths in shrimp, (2) the effects of ocean acidification on statolith structure, (3) the effects of ocean acidification on shrimp's orienting and chemosensory ability, and (4) the effects of ocean acidification on survivorship at multiple life history stages of *Litopenaeus stylirostris* and *Synalpheus carinatus* shrimp, and shrimp from the infraorder *Caridea* and *Stenopodidea*. Specific hypotheses included: (1) the partial and full loss of statoliths will decrease shrimp's ability to orient itself and self-right. (2) Increase in partial pressure of CO₂ and decrease in pH of sea water will cause deterioration of the calcified statoliths and consequently impair balance and orientation ability of shrimp, when compared to shrimp in ambient sea water. (3) The deterioration of the statoliths of shrimp housed in acidified seawater will affect the balance and orientation behavior of shrimp. (4) Shrimp in acidified seawater will take longer to complete a maze designed to test their orienting ability and chemosensory ability to locate a food source. (5) Shrimp eggs housed in acidified seawater will take longer to hatch. (6) Shrimp eggs housed in acidified seawater will also have a lower survival rate. (7) Shrimp larvae exposed to acidified seawater will also have a lower survival rate when compared to those housed in ambient seawater.



Fig 1. Map of Moorea, French Polynesia showing collection sites. (A) Lagoon: 17°29'23.64" S, 149°49'33.65" W. (B) Dock: 17°29'27.07" S, 149°49'34.03" W. (C) Shrimp Farm: 17°31'3.17" S, 149°50'58.37" W.

METHODS

Study site

Four species of shrimp from Moorea, French Polynesia (Fig. 1) were used to discern the various effects of ocean acidification on internal physiology, survival, and behavior. *Stenopodidea* shrimp and *Synalpheus carinatus* were collected from coral rubble in the lagoon in front of the Gump Research Station (Fig. 1, A). *Caridea* shrimp were caught off the Gump Station dock at dusk using small nets (Fig. 1, B). *Litopenaeus stylirostris* shrimp were purchased from a local shrimp farm on Moorea (Fig. 1, C).

Acidified seawater

Currently, many ocean acidification studies model experimental conditions that are framed by the emission scenarios of the Intergovernmental Panel on Climate Change (IPCC 2007). The IPCC predicts an increase in partial pressure of CO₂ to about 800 ppmv in their business-as-usual emission scenario and to about 1400 ppmv in their worse-case emission scenario by the year 2100 (2007). Ocean pH is also predicted to drop between 0.3 and 0.45 pH units during this time from a pH of about 8.1 to a pH of 7.6.

In the experiments conducted during this study, sea water with a partial pressure of CO₂ of between 1200 ppm and 1400 ppm and a pH of 7.77 ± 0.01 was used to mimic the predicted environmental conditions the shrimp will be subjected to if anthropogenic CO₂ emissions are not mitigated. This was maintained by using the Neptune Systems Aquacontroller Apex Lite aquarium control system. Seawater pH was depressed via the bubbling of pure CO₂ through an airstone placed in the flume. Addition of the CO₂ was controlled by a solenoid, which was activated when the aquacontroller detected a pH above the desired value. Seawater pH was elevated by bubbling of ambient air through a through an airstone placed in the flume. Addition of this air was controlled by an aquarium air pump (Fluval Q202) which was activated when the aquacontroller read a pH value below the desired level. The addition of pure CO₂ and

scrubbed air sufficiently maintained pCO₂ levels within 50 ppm of the desired set point. Acidified seawater was collected from flumes and stored in air tight bottles until use in treatments.

Litopenaeus stylirostris: statolith ablations

This study sought to determine the functional importance of statoliths and just how detrimental the loss of one or both statoliths was to basic survival behavior. Many shrimp species possess a self-righting innate behavior, when turned upside down or sideways, they turn themselves over in order to maximize swimming efficiency and swim away from potential predators. Statoliths are believed to play a key role in the self-righting response as it involves detection of orientation within the water column.

To answer this question, large *Litopenaeus stylirostris* shrimp were purchased from the local marine shrimp farm, Crevettes de Moorea (Fig. 1, C) (Horn, 2012). Shrimp were allowed one day to acclimate to new tank and surroundings before their statoliths were either unilaterally ablated on one antennule, or bilaterally ablated on both antennule (Fig. 2). This was done by heating up a sharp



FIG 2. Location of statoliths at base of antennules. (A) Shrimp head showing antennules with right eye removed. (B) Zoom in on exposed statolith within statocysts sac of right antennule. (C) Closer zoom showing sediment grains of statolith.

dissecting probe and destroying the region of the antennules that possess the statoliths. Sham ablations, in which shrimp were stabbed

in non-essential parts of the antennule, were performed to account for the stress of handling and being stabbed. This was also compared to the self-righting time of intact control shrimp. Shrimp were given another day to adjust and distress from ablation procedures before testing. Shrimp of each ablated type were placed in a tank with their dorsal side down and the time it took for them to right themselves was recorded. Both males and females were used and the tank was drained and cleaned between tests to eliminate any chemical queues possibly left by previous shrimp.

Caridea: behavior, statolith structure, and survival

It was hypothesized that acidified seawater would have deleterious effects on the CaCO₃ based statoliths contained within the shrimp's antennules and that the deterioration would then affect orientation and movement abilities. It has also been indicated (Costero and Meyers 1993) that chemoreception is a complex physiological process that may also be disrupted by environmental stressors such as being housed in an irregularly hypercapnic environment. So with that in mind, I wanted to ask how acidified seawater affects chemosensory and orientation ability in shrimp with CaCO₃ statoliths. Shrimp of the infraorder *Caridea*, were caught off the Gump Station dock (Fig. 1, B). 30 shrimp were housed individually in cups of acidified seawater (pH = 7.77 ± 0.01) and 30 shrimp were housed individually in cups of ambient seawater (pH = 8.16 ± 0.03) pumped in from the bay. Cups were stored outdoors under a covered wet lab, exposed to natural ambient temperature and humidity. Water pH was measured and changed every night. Shrimp were replaced when they died until November 3, 2013. Death frequency in each treatment was noted. Individual incubation time in various water treatments was noted. Sex and mass, along with molt frequency of shrimp were also noted. Shrimp that had recently molted were not used in trials. Females with eggs were also used. Larva that hatched from females were used in a separate study to determine the effects

reduced pH has on larval development and survivability.

To determine whether ocean acidification has an effect on statolith structure and orientating behavior and chemoreceptor ability, I built a simple maze to test chemosensory ability to find food source and left-right orienting ability (Fig. 3). Shrimp in acidified and ambient seawater treatments were both tested. Shrimp were starved for three days prior to testing to ensure they would seek out the food source. Dead fiddler crabs, *Uca crassipes*, were used as food bait and was placed at the end of the maze. Shrimp were all tested in ambient seawater and water was changed and tank washed between tests to eliminate any possible chemical queues left behind by previous shrimp. The time it took for shrimp to swim through the maze and find the food was measured. Shrimp were allowed a maximum time of 10 minutes to complete trials. A total of six trials were conducted over a six week period.

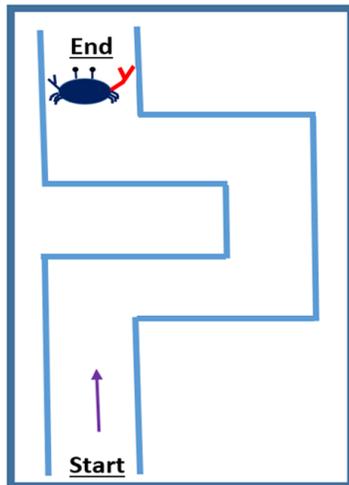


FIG. 3. Diagram of maze *Caridea* shrimp were subjected to in order to determine orienting and chemoreception ability.

To determine if deterioration of existing statoliths was playing a role in shrimp's orientating behavior, statoliths were dissected after the six weeks of incubation and testing, to assess if there was indeed any change in size due to acidified seawater. *Caridea* incorporate sand into their statoliths and in the lagoon of Moorea, French Polynesia, most

of the sand is composed of CaCO_3 from coral rubble, making the question of whether ocean acidification affects the statoliths comprised of CaCO_3 still relevant. Statoliths were dissected and photographed through a dissecting microscope. Body size and statolith diameter, area, and perimeter were measured using ImageJ (Rasband 1997-2012). A ratio of statolith perimeter and body size was then created to account for varying body size, and compared between those housed in acidified conditions, ($\text{pH} = 7.77 \pm 0.01$) and those housed in ambient seawater ($\text{pH} = 8.16 \pm 0.03$). Figure 4 shows an up close photograph of a *Caridea* shrimp's statolith under the microscope.

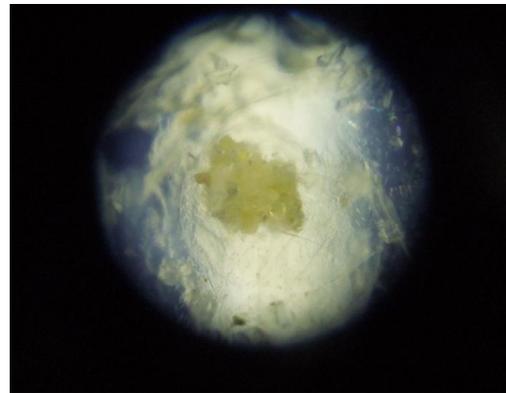


FIG. 4. Dissected *Caridea* shrimp statolith sand grains within statocysts sac.

Synalpheus carinatus, *Caridea*, and *Stenopodidea*: early life history stages

This study also looked at how ocean acidification affects survivorship at early life history stages of shrimp to better understand the whole picture on the outlook of future shrimp populations in the face of climate change. To do this eggs were collected from female *Synalpheus carinatus* snapping shrimp. These snapping shrimp were obtained from coral rubble within the lagoon (Fig. 1, A). 50 eggs were housed in ambient control seawater, 10 eggs per petri dish with a total of 5 petri dishes and 50 eggs were housed in acidified seawater. Water pH was measured was changed each night. The development of eggs was mapped each day until hatching, using photographs and ImageJ (Rasband 1997-2012). Yolk consumption rate

was measured by comparing area of yolk to total area of egg in each treatment. Egg failure was also characterized between treatments. Larval hatchlings were also subjugated to water treatments and their development and survival characterized.

Survivorship in *Caridea* larvae between acidified and ambient water treatments was also determined. Female *Caridea* also carry their eggs on their abdomens, similar to *Synalpheus carinatus*. The clutches used in each treatment were from multiple mothers. Larvae were removed from mother's cup and housed in small groups, between two - five, depending on how large the clutch size was, in separate cups in either acidified or ambient seawater. Water pH was measured and was also changed each night. Larvae were counted and photographed each day to document growth and development and determine survivorship within each treatment.

Larvae from shrimp in the infraorder *Stenopodidea* were also looked at to better determine the effects of ocean acidification on survivorship of shrimp larvae. Here, all larvae in both treatments were from the same mother. Shrimp were identified using photo MBIO13595 from the Moorea BioCode Project database (2010). Shrimp were collected from the lagoon in front of the Gump Research Station (Fig. 1, A). The female specimen had multiple hundreds of larva hatch while in ambient seawater. 100 larva were divided into 25 ambient water cups and 100 larva were divided into 25 acidified seawater cups to determine the effects of acidified water on survivability on the fragile larval stage of this shrimps' life history. Because larvae were from the same mother, it accounted for potential genetic differences and resilience in

the face of pH change.

Analysis

All statistical analyses were conducted in R (R Development Core Team 2013). Analyses of covariance were used to determine the effects of seawater treatment in relation to the number of days shrimp and eggs and larvae were exposed on time to complete the maze and survivorship. Generalized linear model was used in place of ANCOVA if variances between groups were not homogenic. A Tukey HSD test was used to determine which groups significantly differed from the other in the ablation-self-righting tests. Two-sample t-tests were used to determine the effect of acidified and ambient seawater treatment on statolith size and adult *Caridea* death and molt frequency.

RESULTS

Litopenaeus stylirostris: statolith ablations

To determine if the loss of one or both statoliths affected crucial survival behavior *Litopenaeus stylirostris*, generalized linear model was used to determine if body size, along with ablation type, had an effect on self-righting time. It was shown that body size and ablation type did not significantly interact together to influence self-righting time (GLM (quasipoisson), $F_{19} = 0.0057$, $P = 0.94$, Fig. 5) and body size independent of treatment type had no significant effect on self-righting time (GLM (quasipoisson), $F_{20} = 0.012$, $P = 0.91$). The generalized linear model determined that ablation type did have a significant effect on self-righting time (GLM (quasipoisson), $F_{3,35} =$

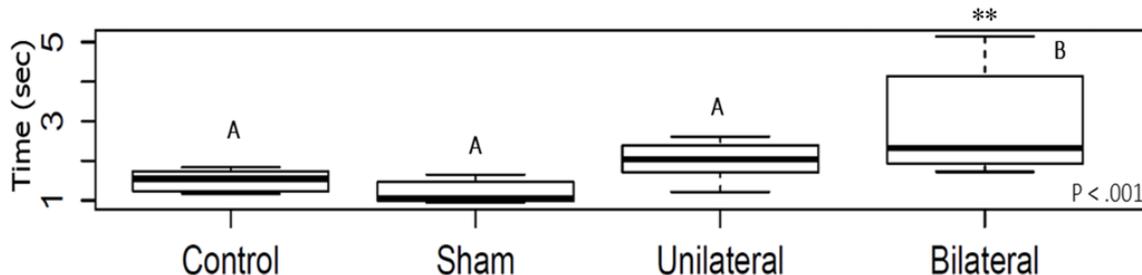


FIG. 5. Boxplot showing distribution of Self-Righting time between each group of statolith-ablated shrimp.

8.58, $P < 0.001$, Fig. 5). A Tukey HSD test was used to determine which treatments were significantly different from each other. Results show that control, sham, and unilaterally statolith-ablated shrimp did not significantly differ from each other in terms of self-right response time, with a mean response time of 1.49 ± 0.08 , 1.14 ± 0.09 , 2.01 ± 0.13 seconds, respectively, when placed in tank ($P > 0.05$). Bilaterally ablated shrimp, with both statolith regions destroyed, showed a significant decrease in self-righting response time when compared to control, sham, and unilaterally ablated shrimp, with a mean response time of 2.81 ± 0.45 seconds (Tukey HSD, $P < 0.001$, Fig. 5).

Caridea: behavior, statolith structure, and survival

Caridea shrimp were housed in either acidified seawater ($\text{pH} = 7.77 \pm 0.01$) or ambient seawater ($\text{pH} = 8.16 \pm 0.03$) for up to 22 days. Throughout their incubation, shrimp were tested in a maze (Fig. 3) to determine the temporal effects of acidified seawater on chemosensory ability and left-right orientating ability. Figure 6 shows the change in time (minutes) between acidified (red) and control (black) treatments and with continued exposure to an acidic environment. Shrimp in control ambient seawater took an average of 1.01 ± 0.1 minutes to finish the maze and find the food source. Shrimp in acidified seawater took an average of 1.72 ± 0.16 minutes to navigate through the maze and detect the food source. An analysis of covariance was used to

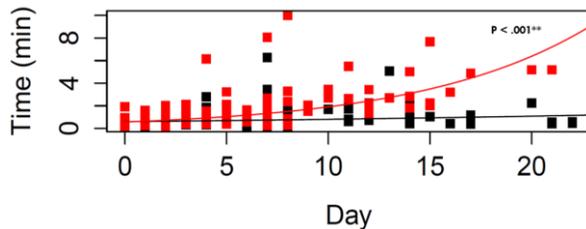


FIG. 6. Time (min) to complete maze and find food source of shrimp in ambient seawater (black) and shrimp held in acidified seawater (red).

determine the effects of water treatment and length of exposure on maze completion time.

Results show that both the number of days exposed to each treatment, and the treatment type and interact together to significantly affect completion time (ANCOVA, $F_{1,201} = 19.86$, $P < 0.001$, Fig. 6). Shrimp in acidified seawater tended to take longer to finish the maze than those in ambient seawater, with those exposed for longer periods of time, up to 22 days, taking the longest on average while the change in time for those in ambient seawater through time is much less.

After maze trials were completed, shrimp in both water treatments were dissected to determine the degree, if any, of statolith degradation correlated with exposure to acidified seawater for extended periods of time (Fig. 7). Body size and statolith diameter, area, and perimeter were measured using imaging software, ImageJ (Rasband 1997-2012). Statolith size was divided by the shrimp's body size to account for discrepancies due to differences in body size. A t-test was run to determine if differences existed between the two treatments. Results show that statolith size decreased in shrimp held in acidified seawater treatment, relative to body size, shown in red, compared to shrimp in ambient seawater (t-test, $Df = 5.3$, $t = -3.34$, $P = 0.019$, Fig. 8). *Caridea* in ambient seawater had an average statolith:body size of 0.26 ± 0.026 , while those in acidified seawater had a ratio of 0.17 ± 0.011 .

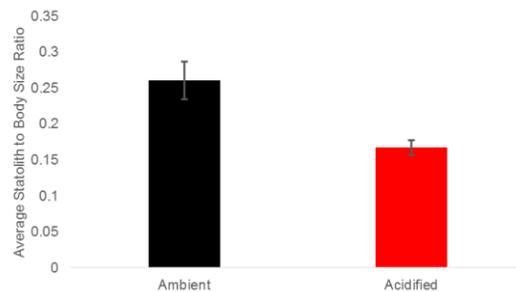


FIG. 7. Average statolith to body size ratio of *Caridea* shrimp housed in ambient and acidified seawater.

The molt frequency and death rate between *Caridea* housed in ambient seawater and those in acidified seawater were not

statistically different (t-test, Df = 36.99, t = -0.095, P = 0.92).

Synalpheus carinatus, *Caridea*, and *Stenopodidea*:
early life history stages

To determine if living in seawater of a reduced pH is detrimental at other life history stages of shrimp, egg development was characterized in *Synalpheus carinatus*. All eggs in both treatments die off with increased time or hatched. Egg survivorship was corrected for those that had developed all the way to hatching. Eggs incubated in acidified seawater (pH = 7.77 ± 0.01) significantly died off at a faster rate than those incubated in ambient seawater (pH = 8.16 ± 0.03), as time increased. An analysis of covariance showed that both days and water treatment interacted to significantly affect survival percentage of shrimp, with those in acidified seawater showing increased mortality over time compared to eggs in ambient seawater (ANCOVA, $F_{1,24} = 1.06$, $P < .001$, Fig. 8).

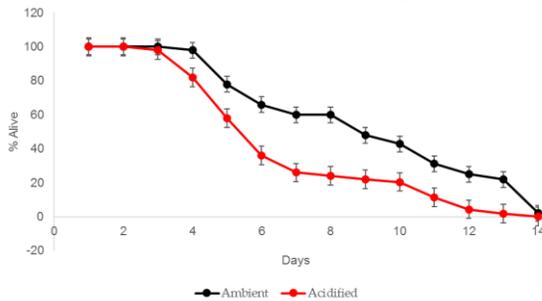


FIG. 8. Percent of eggs alive in ambient and acidified seawater treatments at each time point.

Yolk consumption rate was also looked at to determine the developmental rate of *Synalpheus carinatus* eggs in the different water treatments. A ratio of yolk size to egg size was created to account for other differences in egg morphology. Egg yolk is green and gets smaller as it is consumed and as the egg develops (Fig. 9). An analysis of covariance was used to determine treatment water and the duration of incubation independently had significant effects on yolk consumption (ANCOVA, $F_{1,9} = 10.57$, $P < 0.001$, Fig. 10) and (ANCOVA, $F_{1,9} = 29.95$, $P < 0.001$, Fig. 10) respectively. Egg yolk was consumed at a

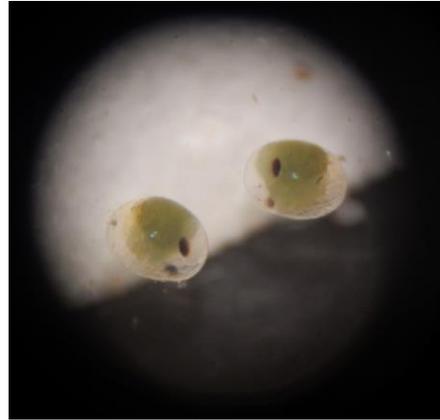


FIG. 9. *Synalpheus carinatus* eggs with green yolk and red eye spots.

faster rate by embryos in ambient seawater (black) than those in acidified seawater (red).

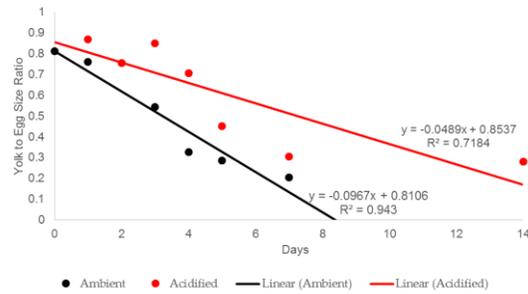


FIG. 10. Yolk consumption rate in eggs incubated in ambient and acidified seawater.

Caridea larvae were used to determine the effects of ocean acidification on larvae survivorship. Figure 11 shows the results from this study. At the beginning, larvae in both treatments begin to die at the same rate but then those in acidified seawater, shown in red, begin to die faster (Fig. 11). Day 6 showed the most prominent difference in terms of survivorship. An analysis of covariance was used to determine if treatment and number of days passed had an effect on survivorship. Results showed that both treatment and days had a significant negative effect on survivorship of shrimp larvae (ANCOVA, $F_{1,10} = 15.81$, $P = .0026$, Fig. 11).

Stenopodidea shrimp larvae were also looked at to further understand the effects of ocean acidification on larvae survivorship and

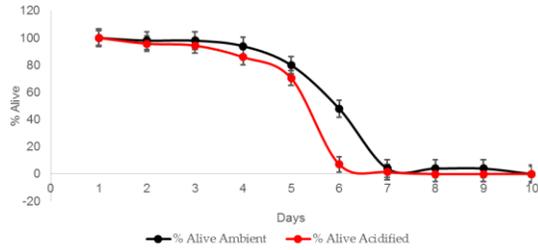


FIG. 11. *Caridea* larvae survivorship in ambient and acidified seawater.

fitness. Once again, survivorship in both groups decreases with time but larvae in acidified seawater treatments (pH = 7.77 ± 0.01), shown in red, die of faster than those in ambient seawater (pH = 8.16 ± 0.03), shown in black (Fig. 12). An analysis of covariance was used to test the effect of water treatment and number of days exposed to treatment on larvae survivorship. Treatment type and number of days interacted together to significantly affect survivorship in *Stenopodidea* shrimp larvae (ANCOVA, $F_{1,38} = 5.20$, $P = 0.03$, Fig. 12).

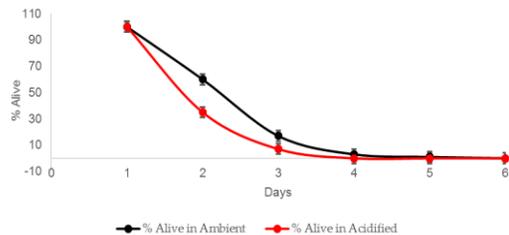


FIG. 12. Larvae survivorship in *Stenopodidea* shrimp in ambient vs acidified seawater.

DISCUSSION

Hatching success and survival

Shrimp larvae, when raised in decreased pH seawater, seem to have decreased survivorship, when compared to those housed in ambient seawater. This difference has been noted in three different shrimp species in this study, *Synalpheus carinatus*, *Caridea*, and *Stenopodidea*. *Synalpheus carinatus* eggs in acidified seawater had decreased survivability than those in ambient seawater. And though eggs in acidified seawater treatment tended to

develop at a slower rate, consuming yolk more slowly, there was no significant difference between earliest hatching day and overall hatching success between the treatments. These findings are similar to previous studies that showed similar disruptions in natural pre-hatching processes caused by changes in environmental pH, though the disruption did not alter overall hatching success (Ceballos-Osuna et al. 2013). The decrease in survivability of shrimp larvae poses a bleak outlook into future shrimp populations as, the early life stages are the most crucial in terms of population dynamics.

Statolith degradation

Most shrimp species possess an innate self-righting response, when turned upside down they are able to re-orient themselves right side up. In my experiment, removing one or both statoliths showed an increase in response time, though unilaterally ablated shrimp self-righting time did not significantly differ from the two control groups. Bilateral ablation of both statoliths showed a significant increase in self-righting response time, indicating that the loss of both statoliths is detrimental to this behavior but the presence of one intact statolith may be enough for *Litopenaeus stylirostris* to self-right and swim away. This study showed that statoliths are an integral part of daily survival behavior in shrimp and the degradation of these key organelles is detrimental at all life stages.

After showing that statoliths possess a functional importance for daily survival behavior, this study aimed to determine if ocean acidification affects statolith structure and the subsequent orienting behaviors associated with them. Similarly, chemoreception was hypothesized to become corrupted and deteriorate in shrimp housed in extreme hypercapnic environments. Olfactory systems are believed to be affected by decreased pH since the chemoreception is a highly sensitive physiological process that has been shown to be negatively affected in other marine organisms housed in low pH seawater (Munday et al. 2009). Here, we showed that a combination of chemoreception and orientation navigating ability was significantly

altered in *Caridea* shrimp housed for extended periods of time in acidified seawater. This decrease in orienting ability may be due to deterioration in statolith structure, which was confirmed by dissecting statoliths of shrimp in both ambient and acidified seawater treatments and analyzing their relative size. It was found that statoliths in shrimp housed in acidified seawater, who took longer to complete the maze and find the food source, also possessed significantly smaller statoliths. This indicates that ocean acidification, to the level tested in this experiment, negatively affects internally housed calcified statoliths and associated navigating and food-finding survival behaviors.

Many studies have focused on the effects of ocean acidification on external calcifying organisms, but few have studied how internal calcified structures such as statoliths are affected by decreased pH. In order to achieve the purpose of this study, all life stages of multiple shrimp species were assessed in terms of mortality and survivorship and physiology in acidified seawater treatments. The importance of statoliths on survival behavior was shown independently from the effects of acidified seawater and then in conjunction with acidified seawater. Degradation in statoliths was assessed and quantified to determine how acidified seawater affects this particular physiology of shrimp.

Growing concerns about the rise in carbon dioxide production has led many to question the numerous effects set about by environmental change. Increases in partial pressure of CO₂ in sea water decreases the saturation level of carbonate based minerals, posing major concerns for calcifying organisms. Many statoliths are secreted of calcium carbonate and are thus susceptible to degradation by acidic ocean conditions. Alterations in statolith structure has been shown to affect shrimps' balance sensory ability. By subjecting shrimp to reduced pH, a relevant and realistic scenario to that predicted by the IPCC, we were able to observe possible outcomes of physiological change and survivability decline in critical life stages of shrimp and the possible implications

for future population dynamics. This study showed that ocean acidification has deleterious effects on survivorship and overall fitness of marine shrimp species.

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