Evidence in Pacific oysters (Crassotrea gigas) of short-term compensatory mechanisms to deal with decreased calcium carbonate availability in acidified conditions

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ABSTRACT

Increasing atmospheric carbon dioxide equilibrates with surface seawater, elevating the concentration of aqueous hydrogen ions. This process, ocean acidification, is a future and contemporary concern for aquatic organisms. Pacific oysters (Crassostrea gigas) were collected from Friday Harbor, Washington, USA (48°31.7’ N, 12°1.1’ W) and spawned in July 2011. Larvae were exposed to three pCO2 treatments: Ambient (400 ppm), MidCO2 (700 ppm), and HighCO2 (1000 ppm). After 24 hours, a greater proportion of larvae in the HighCO2 treatment were calcified as compared to those at Ambient. There was no observed negative impact on larvae held at MidCO2 conditions (700 ppm). However, at 3 days post-fertilization larvae in the HighCO2 environment were smaller and less calcified than controls. The smaller size and decreased calcification at HighCO2 at 3 days can be attributed to developmental delay.

INTRODUCTION

Ocean acidification is expected to affect ecosystems at an accelerating pace over the next century (Caldeira and Wickett 2003; IPCC 2007). Seawater pH declines (acidifies) in association with the uptake of anthropogenic CO2 and resultant increased H+ ion concentration. Projected changes in atmospheric pCO2 may have significant consequences for natural populations ranging from physiological changes to broad-scale range shifts (Talmage and Gobler 2011; O’Donnell et al. 2009; Wong et al. 2011; Tomanek et al. 2011; Banks et al. 2010; Perry et al. 2005).

Acidification of nearshore waters can occur via a variety of processes, including equilibration with elevated pCO2 in the atmosphere, upwelling events, and respiration. The highest concentrations of anthropogenic CO2 are in the near-surface waters of the ocean (Sabine et al. 2004), where larvae in the plankton tend to congregate. The upper ocean acidification in the North Pacific is proportional to the anthropogenic increase in atmospheric CO2, enforcing that the present-day pH changes are outside the range of natural variability (Byrne, R. et al. 2010). In addition to atmospheric sources of CO2, oceanic upwelling and nearshore respiration further reduce the pH of water in which larvae develop (as low as pH 7.4 along the west coast of North America) and increasingly result in waters undersaturated with respect to aragonite (Feely et al. 2008, 2010). These contemporary oceanic processes expose larval C. gigas and other species to transient acidified conditions that have elevated pCO2 similar to projections for future, more sustained conditions. Aragonite is the primary form of calcium carbonate in the shells of many larval molluscs (Weiss et al. 2002) and its undersaturation, specifically due to carbonate ion (CO32-) availability, can hinder shell formation and maintenance in these organisms (Gazeau et al. 2011).

Impacts of ocean acidification have been demonstrated across a range of marine invertebrates and can occur even before fertilization. As CO2 equilibrates in the marine environment can enter gametes and lower intracellular pH, preventing fertilization and development (Kurihara 2008). Low pH has inconsistent effects on fertilization success across taxa of broadcast spawners. Decreased fertilization has been documented in the urchin Heliocidaris erythrogramma (Havenhand et al. 2008), the coral Acropora digitifera (Morita et al. 2009), the sea cucumber Holothuria spp. (Morita et al. 2009), in C. gigas from Australia (Parker et al. 2010), but not in C. gigas from Sweden (Havenhand and Schlegel 2009).

Numerous studies have examined developmental consequences of ocean acidification on marine invertebrate larvae. In two species of urchin, Hemicentrotus pelcherrimus and Echinometra mathaei, larvae exposed to acidified conditions (pH 7.77 and below) demonstrated less early cleavage and a deformed pluteus stage (Kurihara and Shirayama 2004). Similarly in the brittlestar, Ophiothrix fragilis, larvae exposed to pH of 7.9 and 7.7 experienced higher mortality, arrested development before the 8-arm pluteus stage, and a greater proportion of abnormal morphologies than controls (Dupont et al. 2008). The effects of ocean acidification have been studied on populations of C. gigas from Japan (Kurihara et al. 2007), Australia (Parker et al. 2010, 2012), and Europe (Gazeau et al. 2011), but few studies to date look at these effects on populations of C. gigas from the United States. Due to differences in experimental design, it is difficult to directly compare the three aforementioned studies, but overall C. gigas larvae are smaller when raised at elevated pCO2 (Kurihara et al. 2007; Parker et al. 2010; Gazeau et al. 2011), demonstrate a developmental delay (Kurihara et al. 2007), and have morphological and shell deformities (Kurihara et al. 2007; Parker et al. 2010; Gazeau et al. 2011). Pacific oyster larvae are planktotrophs, spending an extended period of one to three weeks in the plankton, where they undergo a variety of important morphological and physiological changes (Strathmann 1985). These developmental changes are frequently associated with environmental cues (Bonar et al. 1990) and their successful completion is necessary for larval metamorphosis into a settled juvenile oyster. Organismal responses to ocean acidification vary among and within taxa suggesting that ecological and evolutionary history may influence responses to ocean acidification. Thus, empirical studies are needed to project responses of species in a given location.

One of the primary means by which marine organisms are directly influenced by ocean acidification is due to relative concentrations of H+ and associated decreased availability of CO32-. These changes in water chemistry impact calcifying organisms as they rely on CO32- to form and maintain carbonate-based structures (Beniash et al. 2010; Thomsen and Melzner 2010), while greater H+ concentration can cause acidosis of body fluids. Acidosis can result in dissolution of calcium carbonate structures, reducing shell thickness and releasing ions into the hemolymph. Many adult aquatic invertebrates can make use of dissolved calcified structures, or possibly actively dissolve their shell, to make HCO3- more available as a buffer against internal acidosis. Excess HCO3- for buffering can also be acquired from the aquatic environment. This phenomenon has been observed in Dungeness crabs Cancer magister (Pane and Barry 2007), blue crabs Callinectes sapidus (Henry et al. 1981), limpets Patella vulgata (Marchant et al. 2010) and urchins Psammechinus miliaris (Miles et al. 2007); however, internal acidosis was not successfully avoided in oysters, C. gigas (Lannig et al. 2010). It is not clear to what degree larvae can utilize this mechanism to maintain homeostasis under elevated pCO2 conditions.

Sustained environmental change, such as ocean acidification, can negatively affect both the ecosystem and economy. Shellfish, including oysters provide important ecosystem services such as improved water quality and benthic-pelagic coupling through the filtration of large volumes of water, release of feces to the benthos, and creation of habitat via reef formation (Coen and Luckenbach 2000). In addition to their ecological roles, molluscs are economically important to many coastal communities worldwide. In 2008, molluscs comprised 64.1% (or 13.1 million tons) of worldwide aquaculture production, with oysters accounting for 31.8% of the total production (FAO 2010). The global economic cost of ocean acidification to the mollusc fishery is unclear but has been estimated to increase with rising atmospheric CO2 levels and terrestrial sources of acidification (Narita et al. 2012). Recently, in the Pacific Northwest of the U.S., concern has heightened over the already apparent effects of corrosive, acidified water on both natural and hatchery production of C. gigas larvae (Elston et al. 2008; Feely et al. 2010;2012). Hatchery These upwelling events have been linked to mortality episodes in the hatchery, perhaps due to a combination of acidic water and pathogens associated with the water masses (Elston et al. 2008). Thus, short-term ocean acidification is a contemporary problem for modern populations.

This study characterized the effects of two elevated levels of pCO2 on size, calcification and development during early larval stages of the Pacific oyster, Crassostrea gigas. Oyster larvae were raised in two elevated levels of pCO2 (700 and 1000 µatm) and ambient (400 µatm) seawater through 72 hours following fertilization. The chemistry scenarios simulated in this study are based on projections for the coming century, but these values of low pH and Ω are already occurring with increasing frequency in nearshore upwelling systems 2009).

MATERIAL AND METHODS

Seawater chemistry manipulation

Experimental conditions were maintained using a flow-through seawater system in Friday Harbor, Washington, USA. Water entering the system was filtered (to 0.2-µm), UV sterilized, and CO2-depleted using membrane contactors (Membrana, Charlotte, North Carolina, USA) under partial vacuum. Three experimental treatments were chosen to correspond with dissolved CO2 levels of 400, 700 or 1000 ppm in the atmosphere. These levels correspond to near current ambient oceanic conditions, projections for mid-century pCO2, and end-of-century, respectively (IPCC 2007). Selected CO2 levels also reflect those currently observed in near and inshore marine waters of the northeastern Pacific (Feely et al. 2008, 2010). These three treatments will be referred to throughout the manuscript as Ambient, MidCO2, and HighCO2. Set-point pH levels were determined with the program CO2SYS (Robbins et al. 2010) using an average total alkalinity of 2060 µmol kg-1 based on total alkalinity measurements taken the week prior to the experimental trial.

Larval C. gigas were held in 3-L microcosms within a large reservoir filled with the respective treatment water. Ambient air stripped of CO2 by a CO2 adsorbtion unit (Twin Tower Engineering, Broomfield, Colorado, USA) was used to aerate the seawater within the reservoirs through a Venturi injector into the larger reservoir of treatment water. This replaced oxygen lost through the degassing process. Reservoir pH was continuously monitored by a Durafet III pH probe (Honeywell, Morristown, New Jersey, USA). When the probe registered that the treatment’s pH strayed from its set point, a solenoid would open or close to allow more or less pure CO2 (Praxair, Danbury, Connecticut, USA) to be injected via the Venturi. The Durafet probe information was fed into a Honeywell UDA2182 pH controller, which also controlled the solenoids.

Seawater was pumped from the reservoir into larval microcosms through irrigation drippers (DIG Industries, Sun Valley, California, USA) at a rate of 1.9-L h-1. An outflow tube at the top of the microcosms fitted with 35-µm mesh allowed water to exit the microcosms while retaining larvae. All systems were equilibrated to the correct treatment level 48 hours prior to the start of the experiment. Water temperature was held at 20.4°C ± 0.4°C

Oysters

Ten female and four male adult C. gigas were collected from Argyle Creek in Friday Harbor, Washington in July 2011. Oysters were strip-spawned into Ambient seawater with eggs and sperm pooled separately (day 0). Pooled eggs (approximately 2 million) were divided equally into 18 7.5-cm diameter containers. Sperm was diluted in Ambient seawater and added to each container of eggs. After the addition of sperm, the eggs were gently agitated and incubated for 15 minutes to allow for fertilization.

Six containers of fertilized eggs were transferred to microcosms containing one of three treatment conditions. Initial densities post-hatching were approximately 1 larva mL-1. On days 1 and 3 post-fertilization, larvae were randomly sampled to determine survival, size, developmental stage, and presence or absence of calcification. For each microcosm sampled, larvae were filtered onto 35-µm mesh screens and washed with the appropriate seawater. Approximately 100 larvae were removed for each sample, relaxed with 7.5% MgCl2 and fixed in 4% paraformaldehyde buffered in filtered seawater. The remaining larvae were returned to cleaned microcosms filled with new seawater. Larvae were fed Dunaliellia sp. and Isochrysis sp. at concentrations of 30,000 cells mL-1 each on day 2. During feeding, water flow was turned off in microcosms for two hours. All microcosms were cleaned at each sampling event.

Larvae were examined using light microscopy to determine survival, size, developmental stage and degree of calcification. Larval hinge length and shell height were measured at 10x magnification with a Nikon Eclipse E600 and NIS Elements Basic Research software (Nikon, Tokyo, Japan). Larval developmental stage and calcification were determined at 20x magnification using an inverted microscope and double polarized light for calcification. All calcification data are qualitative in nature. Larvae were scored as calcified on day 1 post-fertilization if calcification was observed at the hinge (Figure 1A). On day 3 post-fertilization, larvae were classified as fully calcified if polarized light produced a “Maltese cross” in the larval shell (Figure 1B; LaBarbera 1974).

Carbonate chemistry

Salinity was measured with a conductivity meter (Hach sensION5; Loveland, Colorado, USA) and temperature was measured using a Fluke 1523 thermometer (Fluke, Everett, Washington, USA). Seawater pH entering the microcosms was measured daily using the spectrophotometric (spec) technique outlined in SOP 6b by Dickson et al. (2007) to confirm pH measurements from the Durafet probe. When any discrepancies were observed, the Durafet probe was recalibrated. Seawater pH measurements were taken from two microcosms per treatment on days 0, 1, and 3. Final pH values reported here have been corrected for dye addition and temperature. Total alkalinity (AT) was measured following the open cell titration of SOP 3b (Dickson et al. 2007). Samples for AT were taken from incoming water and from two microcosms in each treatment on days 0, 2, and 3. CO2SYS (Robbins et al. 2010) was used to calculate calcium carbonate saturation state (Ω) of aragonite and calcite, carbonate ion concentration, and pCO2 with AT and pH as inputs using the following constants: Lueker et al. (2000) for CO2 Constants, Dickson (1990b) for KHSO4, Total scale (mol kg-1 SW) for pH scale, and Wanninkhof (1992) for Air-Sea Flux.

Statistics

Differences in larval size and mortality across treatments were examined using a two-way ANOVA with fixed effects of treatment and day followed by Tukey’s Honestly Significant Difference test (Tukey’s HSD). A one-way ANOVA was also used to test for differences in larval size among treatments using the combined fixed factor of day-treatment. Larval calcification and developmental stage were compared among treatments using a generalized linear model (GLM). Binomial error distributions were used for GLM analyses. The occurrence of a developmental delay was assessed by fitting the regression of shell height on hinge length to a linear model and testing for differences in the slopes of these lines across treatments. Developmental delay would be demonstrated if the larvae maintained the same allometry across treatments (the slopes of the lines were the same) but were different in size. All analyses were performed in R (R Development Core Team 2011).

RESULTS

Carbonate chemistry

Throughout the experiment seawater pH differed across treatments and AT varied slightly but to the same degree across treatments (Table 1). Mean seawater pH was consistent within but varied among treatments (Figure 2). Mean pH (± standard deviation), as measured by the Durafet pH probes (Figure 2) was 7.99 ± 0.04 in the Ambient treatment, 7.75 ± 0.06 in the MidCO2 treatment and 7.66 ± 0.09 in the HighCO2 treatment. Aragonite and calcite saturation states were greater than 1.0 for the duration of the experiment, except in the HighCO2 treatment on days 1 and 2 (Table 1). Carbonate ion concentration was lowest in the HighCO2 treatment (average ± SD of 61.15 ± 4.05 µmol kg-1 seawater, N = 4, Table 1), intermediate in MidCO2 (74.05 ± 6.43 µmol kg-1, N = 4), and highest in the Ambient treatment (120.24 ± 11.52 µmol kg-1, N = 4). Partial pressure of CO2 in the seawater averaged 468 ± 63 µatm in the Ambient treatment, 847 ± 67 µatm in the MidCO2 treatment, and 1065 ± 58 µatm in the HighCO2 treatment.

Size, development, and calcification

Larval size (shell height and hinge length) was similar across experimental treatments after 24 hours, however by day 3 larvae grew significantly larger (height and length) in the Ambient and MidCO2 ascompared to the HighCO2 treatment (Table 2, Figures 3 and 4). Between days 1 and 3 larvae increased in size under Ambient conditions (shell height, P < 1e-7) and MidCO2 conditions (shell height and hinge length, P < 1e-7 and P = 7.4e-6, respectively; Figures 3 and 4), but did not increase in size under HighCO2 conditions.

Developmental rate did not vary across treatments during the first 24 hours of development, but after day 1 the larvae at HighCO2 experienced a developmental delay. On day 1, a slightly greater proportion (0.977) of larvae at HighCO2 were at the D-hinge stage (compared to those that were still trocophores), but this difference was not significant (z-value=1.016, P = 0.310; data not shown). The proportion of larvae at the D-hinge stage on day 1 in the Ambient treatment was 0.875 and in MidCO2 was 0.833. By day 3, all larvae observed across treatments were at the D-hinge stage. The slope of the linear regression through shell height versus hinge length for the larvae raised at Ambient pCO2 was 0.6459 (Figure 5), which was not significantly different from the slope of the regression line through the MidCO2 data (0.8583, P > 0.05) or from the line through the HighCO2 size data (0.3625, P > 0.10). The similarity in the slopes suggests that the larvae were all following the same growth trajectory and that the larvae raised at HighCO2 were experiencing a developmental delay.

Larval calcification was significantly different among treatments for days 1 and 3 post-fertilization. Following 24 hours of treatment (day 1) the proportion of calcified larvae was inversely proportional to pCO2 level with the greatest number calcified in the HighCO2 treatment (z-value = 2.084, P = 0.0372, Figure 6). On day 3, fewer larvae at HighCO2 conditions were fully calcified compared to the other two treatments (z-value = -3.203, P = 0.00136).

Survival was near 100% in all treatments on day 1 (Ambient = 99.0%, MidCO2 and HighCO2 = 99.7%). On day 3, survival was 92.9% in the Ambient treatment, and was approximately 88.6% in the MidCO2 and 85.6% in the HighCO2 treatment. Mortality was similar among treatments and days (P > 0.05).

DISCUSSION

Oyster larvae raised at HighCO2 did not maintain calcification and growth by 72 hours and were smaller and less calcified than larvae from the other two treatments. Larvae in the HighCO2 treatment were the only ones that experienced ΩAr < 1.0 (Table 1). Numerous species experience decreased calcification at Ω < 1.0 (Kurihara et al. 2007; Miller et al. 2009; 2011; Byrne, M. et al. 2010), although some species are still able to form apparently normal calcified structures in undersaturated conditions (Dupont et al. These results are consistent with other studies of Crassostrea spp. larvae in which elevated pCO2 resulted in decreased growth and shell mineralization (Kurihara et al. 2007; Miller et al. 2009). Kurihara et al. (2007) raised C. gigas to 48 hours post-fertilization at an elevated pCO2 of about 2268 µatm, much higher than pCO2 projected for the coming century, and observed a negative effect on calcification as early as 24 hours post-fertilization. The authors also observed a developmental delay in reaching the D-hinge stage at 48 hours post-fertilization (Kurihara et al. 2007). Since we did not measure growth or calcification in our larvae at 48 hours post-fertilization, we are not able to draw direct comparisons with this time point, but we did observe a developmental delay by 72 hours post-fertilization. Similarly, Crassostrea virginica larvae raised from 72 hours post-fertilization through competency at different pCO2 grew more slowly at elevated pCO2 (560 and 800 µatm) and biomineralized less CaCO3 than controls; however Crassostrea ariakensis showed no effect of pCO2 treatment (Miller et al. 2009). It is likely the observed differences between the studies are related to the much higher pCO2 level used by Kurihara et al. (2007) and species- and population-specific differences in acclimation to ocean acidification

Decreased size and calcification at 72 hours is likely a consequence of developmental delay as evidenced by similar growth trajectories across treatments (Figure 5), in spite of the smaller size of larvae in the HighCO2 treatment. This suggests that change in size is not a direct effect of ocean acidification on shell growth and maintenance. In a study comparing faster growing hybrid C. gigas larvae to slower growing inbred larvae, slower growth was attributed to reduced feeding rate and differing allocation of internal energy reserves for metabolic processes (Pace et al. 2006). It is possible that the stress of elevated pCO2 induces similar physiological changes resulting in a developmentally delayed phenotype. It is difficult to detect developmental delay with complete confidence in studies that do not follow larvae through to settlement. In one such study, larval Strongylocentrotus purpuratus were exposed to elevated pCO2 throughout their larval period and from this perspective it was apparent that ocean acidification caused a delay in development, although at discrete time points this delay could be interpreted as overall smaller size (Stumpp et al. 2011a). Developmental delay may give these species the energetic resources they need to survive stress and reach the later developmental stages of metamorphosis and settlement. However, a delay in development opens the possibility for a host of other complications for pelagic larvae, such as greater potential to be advected to unsuitable habitat (Strathmann 1985), greater chance of being exposed to predators (Underwood and Fairweather 1989), and an overall longer time in the water column where environmental conditions are variable and risky for a free-floating larva.

Smaller larvae, as observed in the highest pCO2 condition in this study, could lead to several ecological disadvantages. Smaller veliger larvae are not able to feed as efficiently as larger individuals (Strathmann and Leise 1979). Larval sand dollars, Dendraster excentricus, responded to increased pCO2 through changes in morphology that resembled a starvation response without the usual compensation of longer arms that allow for greater food capture (Chan et al. 2011). Exposure to ocean acidification altered the larval sand dollar ciliary beat pattern, thus decreasing the efficiency of particle capture, leading to decreased stomach size (Chan et al. 2011). C. gigas larvae also depend on ciliary movement for feeding, although the direct effects of ocean acidification on this mechanism are unknown. In this study we cannot determine if decreased ability to acquire food or less available energy for growth is the main cause for stunted larval size, but these combined impacts of ocean acidification could have additive or synergistic effects on larval growth and development.

A greater percentage of the larvae in the HighCO2 treatment showed evidence of calcification by 24 hours fertilization compared to both Ambient and MidCO2. The greater proportion of calcified larvae initially seen in the HighCO2 treatment is likely evidence of a response to decreased availability of aqueous CO32-. Early C. gigas larval shells are made of amorphous calcium carbonate and aragonite (Weiss et al. 2002), two of the more soluble forms of CaCO3 at low pH. Invertebrates are able to control calcification through amorphous mineral precursors and metabolites (Weiss 2011), thus decreasing the potential effects of a corrosive environment. On days 1 and 2, the time when the larvae in the HighCO2 treatment were beginning to calcify, ΩAr was below 1.0, causing the seawater to be undersaturated with respect to aragonite. The larvae were still able to calcify because calcification occurs in a cellular compartment that minimizes exchange with the external environment and maintains a supersaturation of CaCO3 (Weiner and Dove 2003). Calcification can become energetically costly due to scarcity of CO32- ions in the environment and disruption of ionic gradients of the calcifying compartment form changes in H+. If oyster larvae remove a fixed number of H+ from their calcifying fluid versus maintaining a fixed ratio of extracellular:intracellular H+, then their energy budget would be more taxed during environmental hypercapnia (Ries 2011). If the acidification event had been transient, as they can be in nearshore upwelling systems, the additional calcification at an early stage may have been maintained by the larvae until normal conditions were encountered. However, in situations where exposure to high pCO2 water is longer, calcification and development can be significantly negatively impacted.

In this study, C. gigas tolerated the MidCO2 treatment through 3 days post-fertilization. The lack of negative effects on calcification in the larvae from the MidCO2 treatment suggests that a cut-off of ΩAr < 1.0 is significant in terms of the ability to biomineralize at this time point in development. An elevated pCO2 of 750 ppm (ΩAr of about 1.0) had significant negative effects on hard clam (Mercenaria mercenaria) and bay scallop (Argopecten irradians) larvae after about 3 weeks of exposure as evidenced by decreased survival, development, growth and lipid synthesis (Talmage and Gobler 2011). The comparable exposure conditions in our study (MidCO2) did not have a negative impact over the time period observed. Due to the similarities of carbonate chemistry parameters with Talmage and Gobler (2011), the differential responses observed across species are likely indicative of variability in species, developmental stage tolerances, or length of exposure. Longer experiments in larvae have demonstrated that the negative effects of ocean acidification persist and sometimes worsen in mussels Mytilus californianus (Gaylord et al. 2011), urchins Strongylocentrotus purpuratus (Stumpp et al. 2011 a and b), abalone Haliotis kamtschatkana (Crim et al. 2011), and oysters Crassostrea ariakensis and C. virginica (Miller et al. 2009).

C. gigas larvae demonstrated short-term compensation in the face of environmental stress from ocean acidification in both the HighCO2 and MidCO2 treatments. It is likely that energy resources necessary to maintain calcification, size, and developmental rate under altered environmental conditions may have a negative impact on other physiological processes. Reallocation of energy associated with invertebrate responses to ocean acidification has been shown to affect several processes, including as soft tissue growth (Gaylord et al. 2011; Beniash et al. 2010), scope for growth (Stumpp et al. 2011a), and shell integrity (Gaylord et al. 2011; Melzner et al. 2011). Additional studies are needed to determine if the ability of oyster larvae to deal with elevated pCO2 on a short-term basis impacts other physiological process.

Conclusions

In this study we observed that an acute, 72 hour exposure to end-of-century projections of ocean acidification (HighCO2) has a negative impact on growth and calcification of oyster larvae. More importantly however, this study revealed that moderate changes in seawater chemistry (MidCO2, about 800 µatm, mean ΩAr > 1.19 ± 0.10) did not have an observed significant impact on larvae through 3 days post-fertilization. Even larvae raised at pCO2 greater than 1000 µatm (HighCO2) were able to survive and calcify in these conditions through 24 hours post-fertilization. It appears compensatory mechanisms may help larval oyster acclimatization to transient environmental changes associated with ocean acidification, at least over a short time period. The results of this study in combination with the life history traits of oysters (broadcast spawners with a large number of planktonic larvae) do suggest that certain organisms have the potential to acclimatize and adapt to environmental change. However, in order to effectively evaluate this possibility, future research should focus on characterizing larvae from diverse genotypes and locations as well as assessing any influences that might be experienced later in development.

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FIGURE LEGENDS

Table 1. Water chemistry data for three experimental treatments – Ambient, MidCO2, and HighCO2. Temperature and Durafet pH measurements are averages from each day based on the Honeywell controller logs. Salinity, total alkalinity (AT), and spectrophotometric (spec) pH are point measurements taken each day. Partial pressure of CO2, Ω, and CO32- were calculated from spec pH and AT.

Table 2. Results from post-hoc Tukey’s HSD following ANOVA for comparisons of hinge length and shell height among treatments. The 2-way ANOVA was performed with “treatment” and “day” as fixed effects and the one-way ANOVA was performed with the fixed effect of “day-treatment”.

Figure 1. D-hinge larvae under polarized light portraying calcification at the hinge without a Maltese cross in the shell (A) and full calcification as evidenced by the Maltese cross (B).

Figure 2. Profiles of pH measurements in the three different treatments – Ambient (black), MidCO2 (light gray), and HighCO2 (dark gray). Average pH for the experiment for each treatment is represented by solid lines. The Durafet probes recorded pH measurements every minute.

Figure 3. Larval hinge length on day 1 (white boxplots) and day 3 (gray boxblots). Results are shown for the Ambient treatment (panel A), MidCO2 treatment (panel B), and HighCO2 treatment (panel C). Boxplots contain the middle 50% of the data and dashed lines encompass data within 1.5x the spread of the middle 50%. Open circles represent outliers. Horizontal black bars indicate median values. An asterisk indicates significant differences within a treatment. On day 3, larvae in the HighCO2 treatment were significantly smaller than those in the other two treatments (P < 0.05).

Figure 4. Larval shell height on day 1 (white boxplots) and day 3 (gray boxblots). Results are shown for the Ambient treatment (panel A), MidCO2 treatment (panel B), and HighCO2 treatment (panel C). Boxplots contain the middle 50% of the data and dashed lines encompass data within 1.5x the spread of the middle 50%. Open circles represent outliers. Horizontal black bars indicate median values. An asterisk indicates significant differences within a treatment. On day 3, shell height was reduced in larvae at HighCO2 relative to those raised at Ambient and at MidCO2 (P < 0.01).

Figure 5. Regression of larval shell height on hinge length by treatment and day. Data from larvae raised under Ambient pCO2 conditions are represented by circles, MidCO2 are triangles, and HighCO2 are diamonds. Size data from day 1 are in black and day 3 are in white. The solid line is the regression line for the Ambient data (intercept = 27.47, slope = 0.65), dotted for MidCO2 (intercept = 15.76, slope = 0.82), and dashed for HighCO2 (intercept = 37.10, slope = 0.36). The slopes of all the lines are statistically the same (P > 0.05).

Figure 6. Proportion of larvae calcified exposed to elevated pCO2. Bars represent calcification on day 1 (white) and day 3 (gray). Proportion of larvae calcified are provided from the Ambient treatment (panel A), MidCO2 treatment (panel B), and HighCO2 treatment (panel C). There is a significant difference in calcification among treatments, with the highest proportion of larvae calcified at HighCO2 on day 1 and the fewest larvae calcified in HighCO2 on day 3.