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 pCO_2 treatments: Ambient (400 ppm), MidCO_2 (700 ppm), and HighCO_2 (1000 ppm). After 24 hours, a greater proportion of larvae in the HighCO_2 treatment were calcified as compared to those at Ambient. There was no observed negative impact on larvae held at MidCO_2 conditions (700 ppm). However, at 3 days post-fertilization larvae in the HighCO_2 environment were smaller and less calcified than controls. The smaller size and decreased calcification at HighCO_2 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 CO_2 and resultant increased H^+ ion concentration. Projected changes in atmospheric pCO_2 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 pCO_2 in the atmosphere, upwelling events, and respiration. The highest concentrations of anthropogenic CO_2 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 CO_2, 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 CO_2, 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 pCO_2 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 (CO_3^{2-}) 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 CO_2 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 pCO_2 (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 CO_3^{2-}. These changes in water chemistry impact calcifying organisms as they rely on CO_3^{2-} 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 HCO_3^- more available as a buffer against internal acidosis. Excess HCO_3^- 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 pCO_2 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 CO_2 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; Barton *et al.* 2012). Hatchery water supply comes from adjacent natural bays and when upwelling events occur, the water that enters the hatchery can reach pCO_2 near 1000 µatm (S. Alin, unpublished data; B. Eudeline, pers. comm.). 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 pCO_2 on size, calcification and development during early larval stages of the Pacific oyster, *Crassostrea gigas*. Oyster larvae were raised in two elevated levels of pCO_2 (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 off the U.S. West coast (Feely *et al*. 2010; Hauri *et al*. 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 CO_2-depleted using membrane contactors (Membrana, Charlotte, North Carolina, USA) under partial vacuum. Three experimental treatments were chosen to correspond with dissolved CO_2 levels of 400, 700 or 1000 ppm in the atmosphere. These levels correspond to near current ambient oceanic conditions, projections for mid-century pCO_2, and end-of-century, respectively (IPCC 2007). Selected CO_2 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, MidCO_2, and HighCO_2. Set-point pH levels were determined with the program CO_2SYS (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 CO_2 by a CO_2 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 CO_2 (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% $MgCl_2$ 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 (A_T) was measured following the open cell titration of SOP 3b (Dickson *et al*. 2007). Samples for A_T were taken from incoming water and from two microcosms in each treatment on days 0, 2, and 3. CO_2SYS (Robbins *et al*. 2010) was used to calculate calcium carbonate saturation state (Ω) of aragonite and calcite, carbonate ion concentration, and pCO_2 with A_T and pH as inputs using the following constants: Lueker *et al*. (2000) for CO_2 Constants, Dickson (1990b) for $KHSO_4$, 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 A_T 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 MidCO_2 treatment and 7.66 ± 0.09 in the HighCO_2 treatment. Aragonite and calcite saturation states were greater than 1.0 for the duration of the experiment, except in the HighCO_2 treatment on days 1 and 2 (Table 1). Carbonate ion concentration was lowest in the HighCO_2 treatment (average ± SD of 61.15 ± 4.05 μmol kg^{-1} seawater, N = 4, Table 1), intermediate in MidCO_2 (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 CO_2 in the seawater averaged 468 ± 63 μatm in the Ambient treatment, 847 ± 67 μatm in the MidCO_2 treatment, and 1065 ± 58 μatm in the HighCO_2 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 MidCO_2 as compared to the HighCO_2 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 MidCO_2 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 HighCO_2 conditions.

Developmental rate did not vary across treatments during the first 24 hours of development, but after day 1 the larvae at HighCO_2 experienced a developmental delay. On day 1, a slightly greater proportion (0.977) of larvae at HighCO_2 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 MidCO_2 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 pCO_2 was 0.6459 (Figure 5), which was not significantly different from the slope of the regression line through the MidCO_2 data (0.8583, P > 0.05) or from the line through the HighCO_2 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 HighCO_2 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 pCO_2 level with the greatest number calcified in the

312 HighCO_2 treatment (z-value = 2.084, P = 0.0372, Figure 6). On day 3, fewer larvae at
313 HighCO_2 conditions were fully calcified compared to the other two treatments (z-value =
314 -3.203, P = 0.00136).
315 Survival was near 100% in all treatments on day 1 (Ambient = 99.0%, MidCO_2
316 and HighCO_2 = 99.7%). On day 3, survival was 92.9% in the Ambient treatment, and
317 was approximately 88.6% in the MidCO_2 and 85.6% in the HighCO_2 treatment. Mortality
318 was similar among treatments and days (P > 0.05).
319
320 DISCUSSION
321 Oyster larvae raised at HighCO_2 did not maintain calcification and growth by 72
322 hours and were smaller and less calcified than larvae from the other two treatments.
323 Larvae in the HighCO_2 treatment were the only ones that experienced $\Omega_{Ar} < 1.0$ (Table
324 1). Numerous species experience decreased calcification at $\Omega < 1.0$ (Kurihara *et al*.
325 2007; Miller *et al.* 2009; Crim *et al*. 2011; Gazeau *et al*. 2011; Byrne, M. *et al.* 2010),
326 although some species are still able to form apparently normal calcified structures in
327 undersaturated conditions (Dupont *et al*. 2010; Catarino *et al*. 2011; Yu *et al*. 2011).
328 These results are consistent with other studies of *Crassostrea* spp. larvae in which
329 elevated pCO_2 resulted in decreased growth and shell mineralization (Kurihara *et al*.
330 2007; Miller *et al.* 2009). Kurihara *et al*. (2007) raised *C. gigas* to 48 hours post-
331 fertilization at an elevated pCO_2 of about 2268 µatm, much higher than pCO_2 projected
332 for the coming century, and observed a negative effect on calcification as early as 24
333 hours post-fertilization. The authors also observed a developmental delay in reaching
334 the D-hinge stage at 48 hours post-fertilization (Kurihara *et al.* 2007). Since we did not
335 measure growth or calcification in our larvae at 48 hours post-fertilization, we are not
336 able to draw direct comparisons with this time point, but we did observe a developmental
337 delay by 72 hours post-fertilization. Similarly, *Crassostrea virginica* larvae raised from
338 72 hours post-fertilization through competency at different pCO_2 grew more slowly at
339 elevated pCO_2 (560 and 800 µatm) and biomineralized less $CaCO_3$ than controls;
340 however *Crassostrea ariakensis* showed no effect of pCO_2 treatment (Miller *et al.* 2009).
341 It is likely the observed differences between the studies are related to the much higher
342 pCO_2 level used by Kurihara *et al*. (2007) and species- and population-specific
343 differences in acclimation to ocean acidification
344 Decreased size and calcification at 72 hours is likely a consequence of
345 developmental delay as evidenced by similar growth trajectories across treatments
346 (Figure 5), in spite of the smaller size of larvae in the HighCO_2 treatment. This suggests
347 that change in size is not a direct effect of ocean acidification on shell growth and
348 maintenance. In a study comparing faster growing hybrid *C. gigas* larvae to slower
349 growing inbred larvae, slower growth was attributed to reduced feeding rate and differing
350 allocation of internal energy reserves for metabolic processes (Pace *et al.* 2006). It is

possible that the stress of elevated pCO_2 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 pCO_2 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 pCO_2 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 pCO_2 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 HighCO_2 treatment showed evidence of calcification by 24 hours fertilization compared to both Ambient and MidCO_2. The greater proportion of calcified larvae initially seen in the HighCO_2 treatment is likely evidence of a response to decreased availability of aqueous CO_3^{2-}. Early *C. gigas* larval shells are made of amorphous calcium carbonate and aragonite (Weiss *et al*. 2002), two of the more soluble forms of $CaCO_3$ 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 HighCO_2 treatment were beginning to calcify, Ω_{Ar} 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 $CaCO_3$

(Weiner and Dove 2003). Calcification can become energetically costly due to scarcity of CO_3^{2-} 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 pCO_2 water is longer, calcification and development can be significantly negatively impacted.

In this study, *C. gigas* tolerated the MidCO_2 treatment through 3 days post-fertilization. The lack of negative effects on calcification in the larvae from the MidCO_2 treatment suggests that a cut-off of $\Omega_{Ar} < 1.0$ is significant in terms of the ability to biomineralize at this time point in development. An elevated pCO_2 of 750 ppm (Ω_{Ar} 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 (MidCO_2) 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 HighCO_2 and MidCO_2 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 pCO_2 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 ($HighCO_2$) has a negative impact on growth and calcification of oyster larvae. More importantly however, this study revealed that moderate changes in seawater chemistry ($MidCO_2$, about 800 µatm, mean $\Omega_{Ar} > 1.19 \pm 0.10$) did not have an observed significant impact on larvae through 3 days post-fertilization. Even larvae raised at pCO_2 greater than 1000 µatm ($HighCO_2$) 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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639 FIGURE LEGENDS
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641 Table 1. Water chemistry data for three experimental treatments – Ambient, MidCO_2,
642 and HighCO_2. Temperature and Durafet pH measurements are averages from each day
643 based on the Honeywell controller logs. Salinity, total alkalinity (A_T), and
644 spectrophotometric (spec) pH are point measurements taken each day. Partial pressure
645 of CO_2, Ω, and CO_3^{2-} were calculated from spec pH and A_T.
646
647 Table 2. Results from post-hoc Tukey's HSD following ANOVA for comparisons of hinge
648 length and shell height among treatments. The 2-way ANOVA was performed with
649 "treatment" and "day" as fixed effects and the one-way ANOVA was performed with the
650 fixed effect of "day-treatment".
651
652 Figure 1. D-hinge larvae under polarized light portraying calcification at the hinge
653 without a Maltese cross in the shell (A) and full calcification as evidenced by the Maltese
654 cross (B).
655
656 Figure 2. Profiles of pH measurements in the three different treatments – Ambient
657 (black), MidCO_2 (light gray), and HighCO_2 (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), MidCO_2 treatment (panel B), and HighCO_2 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 HighCO_2 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), MidCO_2 treatment (panel B), and HighCO_2 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 HighCO_2 relative to those raised at Ambient and at MidCO_2 ($P < 0.01$).

Figure 5. Regression of larval shell height on hinge length by treatment and day. Data from larvae raised under Ambient pCO_2 conditions are represented by circles, MidCO_2 are triangles, and HighCO_2 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 MidCO_2 (intercept = 15.76, slope = 0.82), and dashed for HighCO_2 (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 pCO_2. Bars represent calcification on day 1 (white) and day 3 (gray). Proportion of larvae calcified are provided from the Ambient treatment (panel A), MidCO_2 treatment (panel B), and HighCO_2 treatment (panel C). There is a significant difference in calcification among treatments, with the highest proportion of larvae calcified at HighCO_2 on day 1 and the fewest larvae calcified in HighCO_2 on day 3.