AN ABSTRACT OF THE THESIS OF

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Abstract approved:

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George Waldbusser

Atmospheric carbon dioxide has increased by approximately 40% since preindustrial times from 280 ppm (parts per million) to 400 ppm. The rise in Pco_2 is causing the ocean to become more acidic due to the uptake of carbon dioxide by the water, termed "ocean acidification". As the Pco_2 in the ocean increases, the pH and the saturation state, Ω, of calcium carbonate decrease. Increased Pco_2 in the ocean has been shown to negatively affect calcifying organisms like the Pacific oyster (*Crassostrea gigas*). The effects of elevated Pco_2 on egg size and 24-hour old, D-hinge larval size and development were examined in this study. Adult Pacific oysters were conditioned in low and high levels of Pco_2. From these groups, two treatment groups were made for larval rearing, high and low Pco_2. Significant decreases in egg size and normal larval development were found due to the adult treatment. Larval size differed significantly between adult exposure treatments. These results suggest the negative effects of ocean acidification can be carried over from adult to offspring, with egg size acting as a mechanism. The evidence of carryover effects supports the study of ocean acidification across multiple life history stages rather than a single phase.

Key Words: ocean acidification, calcification, bivalves, carryover

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Ocean Acidification Carryover Effects from Oyster Broodstock to Larvae

by

Victoria G. Klein

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I understand that my project will become part of the permanent collection of Oregon State University, University Honors College. My signature below authorizes release of my project to any reader upon request.

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Victoria G. Klein, Author

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DEDICATION

This work is dedicated to my loving parents, Bill and Jill, who have shown me the importance of hard work, compassion, enthusiasm, and always striving to do your best. I would not be the person I am today without them.

OCEAN ACIDIFICATION CARRYOVER EFFECTS FROM OYSTER BROODSTOCK TO LARVAE

INTRODUCTION

Since the industrial revolution, the partial pressure of carbon dioxide (Pco_2) in the atmosphere has been rising (IPCC 2011). Recently, measurements at the Mauna Loa Observatory show that atmospheric carbon dioxide has reached a record level of 400ppm (Ewald 2013). The ocean acts as carbon sink in the global carbon cycle, so the amount of carbon dioxide in the ocean increases as the amount of atmospheric carbon dioxide increases (Sabine et al. 2004). While this uptake of carbon by the ocean is beneficial in reducing the greenhouse warming of the earth by removing carbon from the atmosphere, this same uptake has significant impacts on the water chemistry of the ocean (Barker and Ridgewell 2012). When carbon dioxide dissolves in seawater it forms carbonic acid (H_2CO_3) which can lose hydrogen ions (H^+) and dissociate to bicarbonate (HCO_3^-) and carbonate (CO_3^{2-}) ions. Increasing the amount of carbon dioxide in the ocean increases the concentration of aqueous carbon dioxide, bicarbonate, and hydrogen ions while causing a decrease in the number of carbonate ions freely available in the water (Doney et al. 2009; Barker and Ridgewell 2012). The increase in hydrogen ions leads to a decrease in ocean pH according to the inverse logarithmic relationship between pH and hydrogen ion concentration ($pH = -\log_{10}[H^+]$). A 2007 IPCC report forecast that based on a best available scenario the pH of the ocean will decrease by 0.3 to 0.4 units by the end of the 21st century which corresponds to a 150% increase in hydrogen ion concentration and a 50% decrease in carbonate ion concentration (Orr et al. 2005).

The reduction in available carbonate ions has negative biological implications for calcifying marine organisms like coral species, coralline algae, mollusks, and many other marine invertebrates (Fabry et al. 2008; Doney et al. 2009; Barker and Ridgewell 2012). Many of these

calcifying organisms build calcareous (calcium carbonate containing) shells or skeletal structures (Fabry et al. 2008). This biomineralization process is highly dependent on the saturation state (Ω) which is defined as:

$$\Omega = [Ca^{2+}][CO_3^{2-}]/ K'_{sp}$$

in which K'_{sp} refers to the apparent solubility of calcium carbonate in the seawater which is dependent on temperature, pressure, and salinity of the water (Doney et al. 2009). As the carbonate ion (CO_3^{2-}) concentration decreases with decreasing ocean pH, the saturation state of the seawater decreases as well. In supersaturated conditions ($\Omega > 1$), there is an excess of [CO_3^{2-}] in the water allowing calcium carbonate crystals to grow. Conversely, in undersaturated conditions ($\Omega < 1$), there is a dearth of carbonate ions in the seawater, causing calcium carbonate to dissolve because the dissolved state is more thermodynamically favorable (Barker and Ridgewell 2012; Barton et al. 2012). When the ocean is in this undersaturated state, calcifying organisms, especially calcium carbonate shell-forming bivalves like the Pacific oyster (*Crassostrea gigas*) (Gazeau et al. 2007), are negatively affected. The larval stage of this organism and many other marine organisms is particularly vulnerable to decreases in saturation state (Parker et al. 2009; Barton et al. 2012). One of the hypothesized reasons for greater larval sensitivity to the decreased saturation state is presence of amorphous calcium carbonate (ACC) in the larval shells (Parker et al. 2013). ACC is the form of calcium carbonate that is deposited during early larval development; it lacks a crystalline structure, which makes ACC more susceptible to dissolution (Weiss et al. 2002; Wilt 2005; Wicks and Roberts 2012).

Other life history stages of marine organisms have also been found to be affected by elevated levels of Pco_2 in the ocean. Kurihara et al. (2008) found that egg production in the shrimp *P. pacificus* was suppressed after long periods of exposure to elevated Pco_2 levels. Several studies have also shown that increased Pco_2 negatively impacts fertilization success (*H. erythrogramma*, Havenhand et al. 2008; *S. glomerata* and *C. gigas*, Parker et al. 2009, 2010).

While there are many studies that focus on the effects of increased Pco_2 in the ocean on the individual life history stages of marine organisms, there are far fewer studies that focus on the connections between these life history stages. In 2012, using the Olympia oyster (*Ostrea lurida*), Hettinger et al. studied the potential for the effects of ocean acidification to be carried over from the larval to the juvenile stage. Oysters were first reared through their larval stage in ambient and lower pH environments, and then these oysters were reared through their juvenile stage in both the ambient and lower pH environments. This study found evidence of a "strong carry-over effect from the larval phase" to the juvenile phase made apparent from the decrease in shell growth rate regardless of the pH level to which the juveniles were exposed. Similarly, Parker et al. (2012) examined the relationship between the life history stages of the Sydney rock oyster (*S. glomerata*) by reproductively conditioning broodstock in elevated and ambient Pco_2 conditions. The larvae from these oysters were reared in the same environment as the broodstock while the other larvae were reared in the opposite Pco_2 environment; that is, larvae from broodstock reproductively conditioned in elevated Pco_2 were reared in ambient Pco_2 and vice versa. By focusing on multiple life history stages, Parker et al. (2012) found that the Sydney rock oyster might have the ability to acclimate or adapt to changing conditions as the larvae from oysters conditioned in elevated Pco_2 did better in the elevated Pco_2 treatment than the larvae from the oysters conditioned in ambient Pco_2.

The purpose of the study presented here was similar to that of Hettinger et al. (2012) and Parker et al. (2012). Like the Sydney rock oyster used by Parker et al. (2012), the Pacific oyster (*C. gigas*) is a commercially important species, so understanding the effected ocean acidification has on this species is crucial. In this study, Pacific oysters were reproductively conditioned to see how egg size, larval growth, and larval development are affected by high levels of Pco_2. These two life history stages were chosen for two reasons. First, the larval stage seems to be particularly vulnerable to Pco_2 changes (Kroeker et al. 2010; Barton et al. 2012). Secondly, egg size has been suggested to affect fertilization success in broadcast spawning marine invertebrates (Levitan

2006), and has been correlated to the potential fitness of a larvae due to possible higher energy content in the egg (McEdward and Morgan 2001). The goal of this study is to examine the effects of ocean acidification carried over from broodstock through eggs to the D-hinge larval stage.

METHODS

Collection and Exposure of Adults

Pacific oysters, *Crassostrea gigas*, were harvested in August of 2012 from Oregon Oyster Farms in Newport, Oregon. Seventy-two representatives of this population were collected (ash-free dry weight mean = 1.06 g ± 0.22) and transferred to the Hatfield Marine Science Center lab in Newport, Oregon. Oysters were cleaned to remove sediments and other organisms. Once cleaned, the oysters were divided into twelve 10-L buckets. These 10-L buckets were part of a flow through system in which sea water, filtered through a sand filter then through 10μm filter bags, entered two 50-gallon head tanks, each of which was connected to 6 of the smaller buckets containing 6 oysters each. The flow rates of these buckets ranged from 0.529ml/sec to 1.20mL/sec (mean ±StDev = 0.816mL/sec ± 0.236). The two head tanks represented the two Pco_2 treatments were used in this study, a "high" Pco_2 tank and a "low" Pco_2 tank. The target Pco_2 levels were 1600ppm and 400ppm for the high and low treatments, respectively.

The treatment conditions were maintained by bubbling gas into the two head tanks. The gas bubbling was controlled and maintained by using a feedback pH system. The low tank was maintained by continuously bubbling air from outside the building into the tanks. The high Pco_2 tank was controlled by additions of carbon dioxide gas. The gas was delivered to the head tanks by a thin tube attached to an air stone at the bottom of the tank to ensure even mixing. The pH levels of the water in the two head tanks were continuously monitored by the computer on an aquarium AquaController. The monitoring system was set for a target pH. In the high tank, if the pH of the target went 0.1 units above the target pH the CO_2 gas was turned on by a solenoid valve and if the pH went 0.1 units above the target pH the CO_2 gas was turned off using the same solenoid valve. The pH was recorded by the pH electrode connected to the computer every 5

minutes and samples of the water were taken from the head tanks daily, stored in amber bottles and fixed with HgCl, to be analyzed later for Pco_2 level (Hales et al. 2005).

The adult oysters were conditioned in these treatment tanks for one week. The water temperature was maintained between 18°C – 20°C for the week.

Collection of gametes, fertilization, and stocking

Following the week of conditioning in the treatment waters, oysters were strip spawned. This was chosen over the traditional natural spawning method, in which oysters are put through heating and cooling cycles to induce spawning, to avoid shock response to either change in temperature or water chemistry which could have an effect on the health of the gametes. To remove the gametes, oysters were sorted based on male and female then their gonads were scored with a razor blade then rinsed into a beaker of treatment water (O'Conner et al. 2008). To remove debris, the water from this sample was decanted through a 100-µm sieve and the eggs were on a 20- µm sieve. The eggs were allowed to hydrate in their respective treatment waters for 15 minutes. A sample of eggs was taken from the two adult treatments and preserved for egg size measurements. The sperm was also filtered to remove any debris or gonad tissue. The sperm mixture was poured through a 20-µm sieve and the suspension that ran through the sieve was collected. The eggs and sperm from the 6 female and 3 male individuals were combined in a beaker of treatment water. The mixture of gametes was allowed to sit on the lab bench for 4 hours, and then the eggs were checked for fertilization success. This was done by examining a sample of the fertilized eggs for evidence of polar bodies on about 90% of the eggs which are an early indication of cellular division.

Following conformation of fertilization, the mixture of eggs and sperm was filtered through a 20-µm sieve to collect the fertilized eggs and remove any unfertilized eggs and unused sperm. Fertilized eggs were then stocked into twenty 500mL glass bottles at an initial stocking

concentration of 4 eggs/mL for a total of 2000 fertilized eggs per treatment bottle. At this point, there were 4 treatments created. Fertilized eggs from the oysters conditioned in high Pco_2 water were put into the glass bottles, half of which had low levels of Pco_2 and half with high Pco_2. These treatments are later referred to as Hh and Hn, respectively. The adult treatment is signified by the capital letter, and the lower case letter refers to the larval treatment with h for high and n for low. The same was done for the fertilized eggs from the oysters conditioned in the "low" Pco_2 water which were called Nh and Nn (Figure 1). These bottles (4 treatments x 5 bottles per treatment = 20 total bottles) were allowed to incubate for 24 hours in a 20°C room.

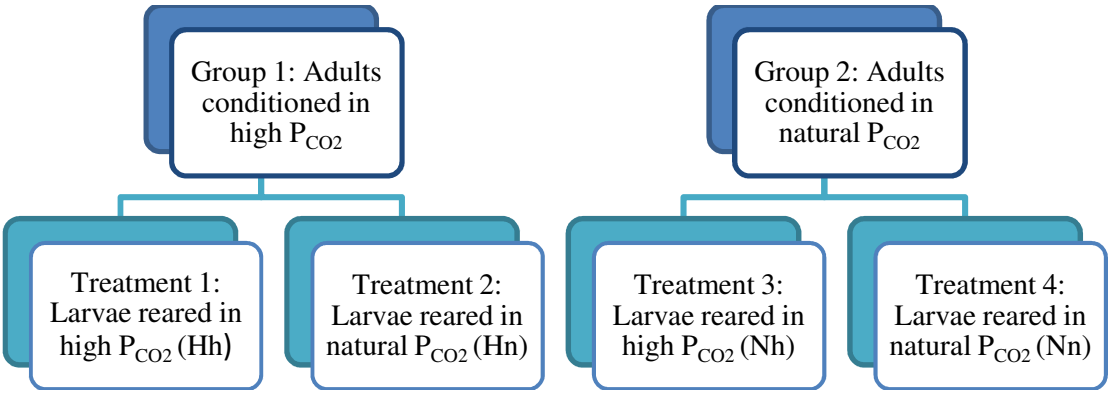


Figure 1: Flow chart showing the experimental setup for this study. There were two broodstock conditioning groups, high Pco_2 and low Pco_2. The fertilized eggs from each of these groups were then divided into two groups high Pco_2 and low Pco_2 which makes 4 total treatment groups. The two letter codes (Hh, Hn, Nh, and Nn) represent the treatment names. The first letter represents the group in which the broodstock were conditioned (H for high and N for low). The second letter represents the group in which the larvae were reared (h for high and n for low).

After this 24 hour period, water chemistry samples were taken from each of the 500mL glass bottles using Viton tube siphon lines, with 37 µm mesh around the mouth of the siphon, to measure pH, saturation state, and Pco_2. These samples were stored in amber bottles with 30 µL of HgCl in the same method used during the conditioning period. The remaining treatment water was poured into a beaker and the mass of the water in the beaker was measured to determine the volume of water remaining, since the density of water is approximately 1 g/mL. While

homogenizing this water and larvae mixture in the beaker, four 5mL samples were taken from each of the beakers. Each of these samples was fixed with 50 µL of buffered formalin. The total number of larvae in the treatment bottle was determined by extrapolating the average number of larvae in the 5mL samples to find the number in the volume of treatment water that was poured into the beaker.

Methods of Analysis

Pictures of the larvae and eggs were taken using an inverted compound microscope attached to a digital camera. A picture of a 1 mm scale bar was also taken, and the image analysis software ImageJ (version 7) was used to measure the length and height of the larvae (Figure 2) and the planar area of the eggs. This provides a good proxy for egg quality, as larger eggs tend to be higher in quality as they have higher lipid counts (McEdward and Morgan 2001). Oyster larvae were also counted to determine hatching success and the percent of normally developing larvae. Hatching success was determined by comparing the number of fertilized eggs that hatched in a sample compared to the initial stocking density of fertilized eggs per treatment bottle. The oyster larvae were divided into three qualitative categories based on shell development: normal (straight hinge, D-shaped shell), abnormal (non-straight hinge, but still mostly D-shaped shell), and fair to poor (hinge not straight at all and/or shell more oval than D-shaped when present at all) (Figure 2). The percent normal was assessed counting the number of normal larvae in each sample and comparing that to the estimated total number of larvae in that sample.

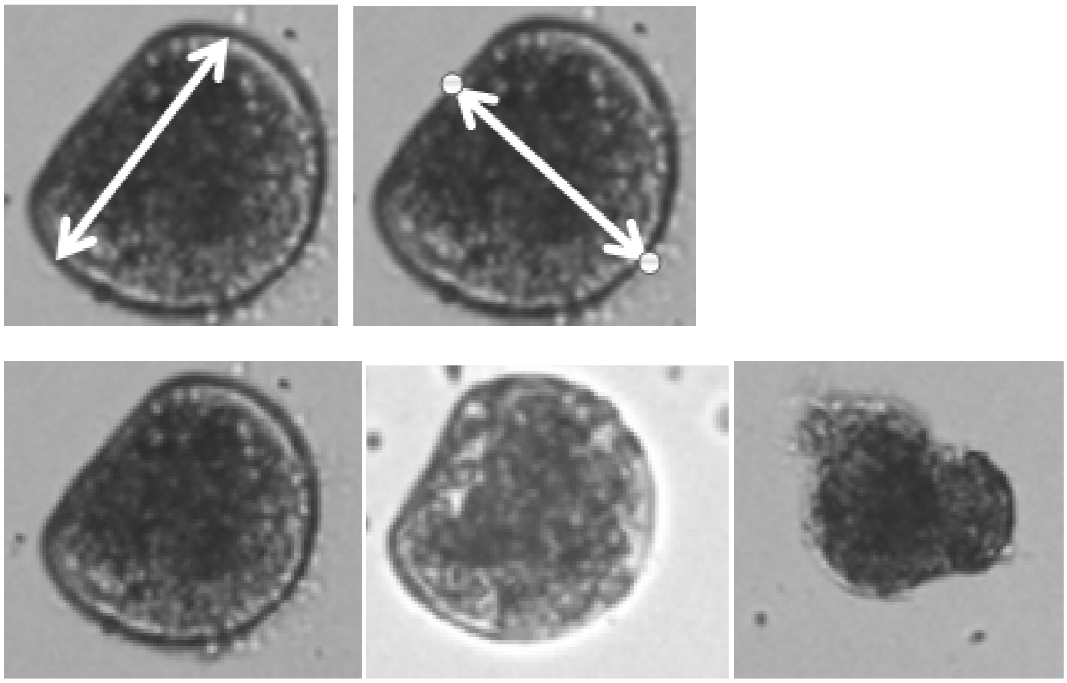


Figure 2: Example images of 24-hour old larvae. *Top:* White arrows on these pictures show how shell length (left) and shell height (right) were measured. Shell length is defined as the longest distance from edge to edge parallel to the hinge, and shell height is defined as longest distance from the hinge to the opposite edge of the shell perpendicular to the hinge. *Bottom:* from right to left, representative images of normal D-hinge larvae, abnormal D-hinge larvae, and fair to poor D-hinge larvae for *C. gigas* that were used to qualitatively compare the developmental success of larvae from each of the four treatments.

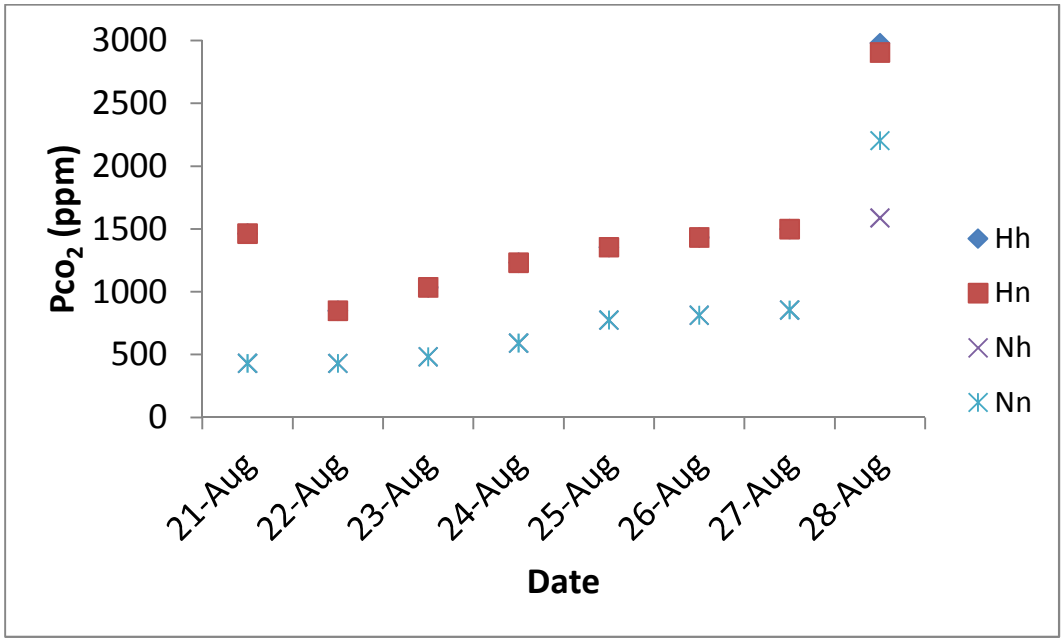
A one-way ANOVA in Minitab was also used to confirm that there was a significant difference in the Pco_2 and the saturation state of the two treatment tanks. A one-way ANOVA test was used to determine whether there was a significant difference in egg size. Fifty eggs from each treatment were used to for this test. For the hatching success, percent normal, shell length, and shell height, two-way ANOVA tests with Tukey tests were used to test for significant differences between the treatments. The two fixed factors in the two-way ANOVAs were adult exposure and larval exposure. For hatching success, percent normal, and shell length and height calculations, the ANOVA was run using 5 values per treatment (Hh, Hn, Nh, and Nn), the averages of the four 5mL samples for each of the 5 treatment bottles for the treatments. For shell length and height, the number of larvae per bottle within each treatment was between 26 and 73, with an average of

about 42 larvae. The statistical tests described above were performed using SAS unless otherwise noted. No larvae developed in one of the Hn bottles, possibly due to an error while adding fertilized eggs to the treatment bottles during the stocking phase. For this reason, the sample was excluded from the analysis.

RESULTS

Carbonate Chemistry

Based on the bottle samples, the "high" tank averaged 1267.4 ppm ± 243.8 (mean ± StDev) and the "low" had an average Pco_2 of 626.4 ± 186.2 (mean ± StDev). The "low" tank was higher than expected. This is likely because the flow rate was too fast to allow for the air being bubbled into the tank to reduce the Pco_2 of the tank. The conditioning tank with high Pco_2 water had significantly higher Pco_2 ($F_{1,12} = 30.57$, $p<0.001$), lower pH ($F_{1,12} = 29.47$, $p < 0.001$), and lower saturation state ($F_{1,12} = 23.13$, $p<0.001$) than the tank with low Pco_2 water (Figure 3). After the 24-hour rearing period, there was an increase in the Pco_2 level and a decrease in the saturation state of the treatment bottles containing the developing larvae. By the end of the 24-hour rearing period, the two treatments with larvae from adult oysters conditioned in high Pco_2 water had similar Pco_2 ($F_{1,8} = 0.31$, $p=0.591$) and saturation states ($F_{1,8} = 0.57$, $p=0.473$), and the same is true for the two treatments with larvae from adult oysters conditioned in low Pco_2 water (Pco_2: $F_{1,8} = 0.84$, $p=0.386$; saturation state: $F_{1,8} = 1.30$, $p=0.287$) (Figure 3).



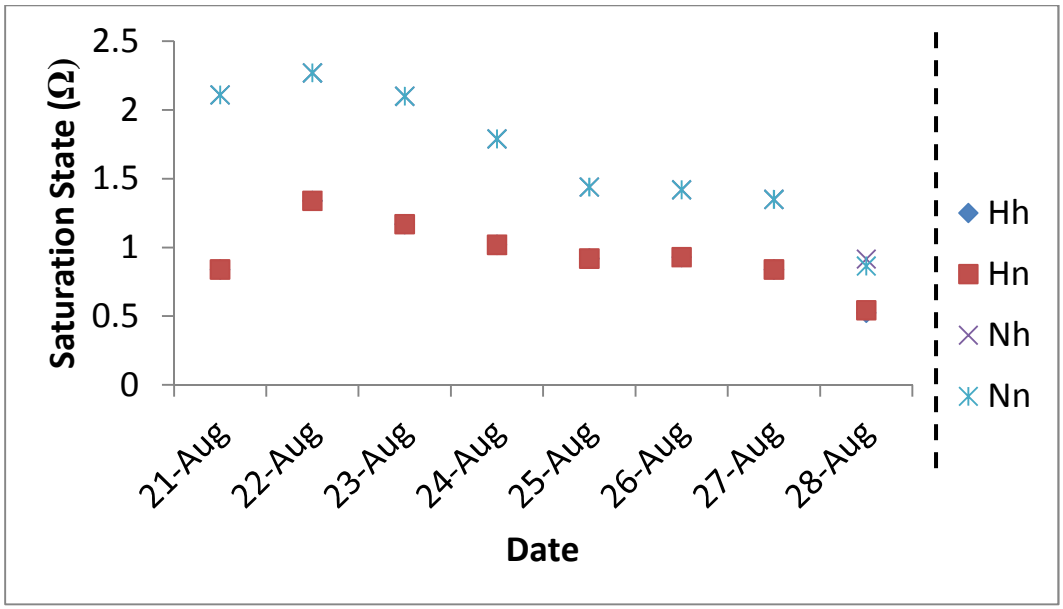


Figure 3: The Pco_2 (top) and the saturation state (bottom) data from the daily water samples taken in amber bottles for the broodstock conditioning period and the larval exposure period. The 7 days to the left of the dotted line (21-Aug through 27-Aug) represent the broodstock conditioning period. Broodstock conditioning stopped on the 27th, and larval exposure began on the 27th. The date to the right of the dotted line (28-Aug) represents the water conditions of the treatment water after 24 hours of larval exposure. The Hh diamond symbol is below the Hn square symbol on the graph.

Egg Size

The quality of the oyster eggs was evaluated based on their planar area. The adult treatment had a significant effect on the growth on the egg size. Eggs harvested from oysters conditioned in the high Pco_2 treatment had significantly smaller areas than the eggs harvested from oysters conditioned in the low Pco_2 water ($F_{1,98} = 23.2$, $p < 0.0001$, Table 1 and Figure 4).

Table 1: Table showing the ANOVA results for egg sizes

Factor	Numerator Degrees of Freedom	Denominator Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Adult Treatment	1	98	0.005	0.005	23.2	<0.0001
Residual	98		0.022	0.0002		

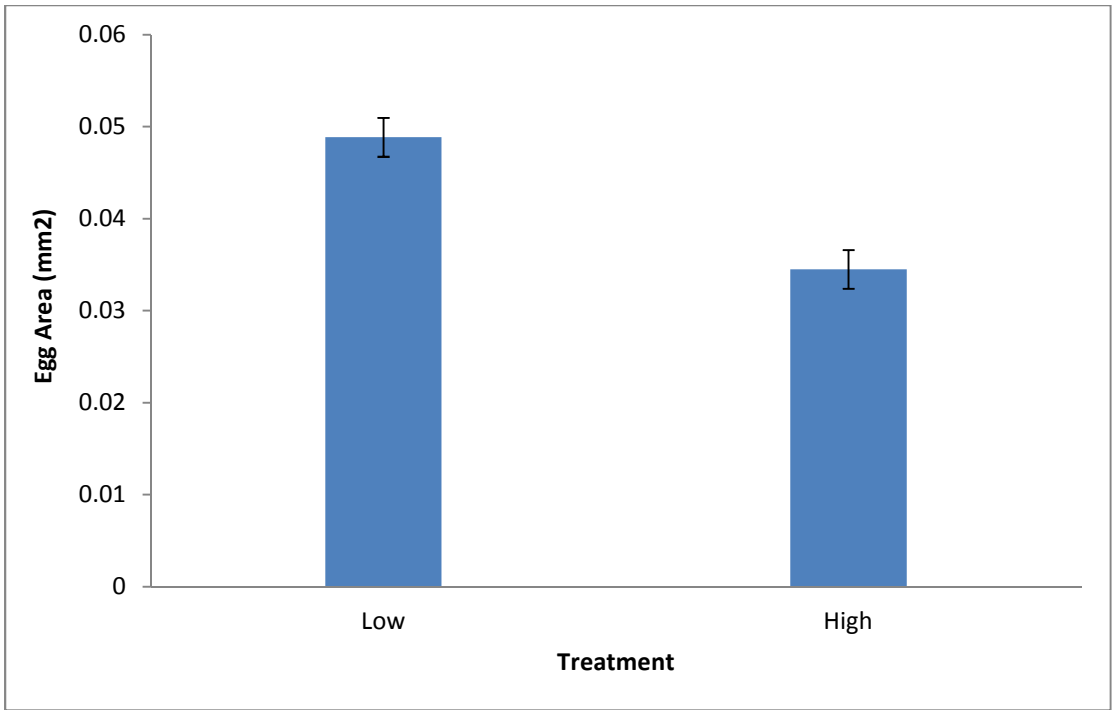


Figure 4: Comparison of egg sizes (planar area) harvested by strip spawning from petite *C. gigas* broodstock conditioned for one week at high and low Pco_2 in a flow through system. "High" refers the oysters that were conditoned for one week in high Pco_2 water (on average 1267ppm). "Low" refers the oysters that were conditoned for one week in high Pco_2 water (on average 626ppm). Error bars are the standard error.

Hatching Success and Percent Normal Larvae

For hatching success, it was found that there was no statistical difference among the four treatments (by adult exposure: $F_{1,16} = 1.94$, $p = 0.1823$; by larval exposure: $F_{1,16} = 2.16$, $p = 0.1606$; Table 2). However, there were about 500 to 600 more successful hatchings out of 2000 fertilized eggs in the treatment in which both the adults and larvae were in low Pco_2 water than in the other three treatments in which the broodstock, larvae, or both were conditioned in high Pco_2 water (Figure 5).

Table 2: Table showing the ANOVA results for the hatching success

Factor	Numerator Degrees of Freedom	Denominator Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Adult	1	16	307582	307582	1.94	0.1823
Larval	1	16	342519	342519	2.16	0.1606
Interaction	1	16	456676	456676	2.89	0.1087
Residual	16		2531351	158209		

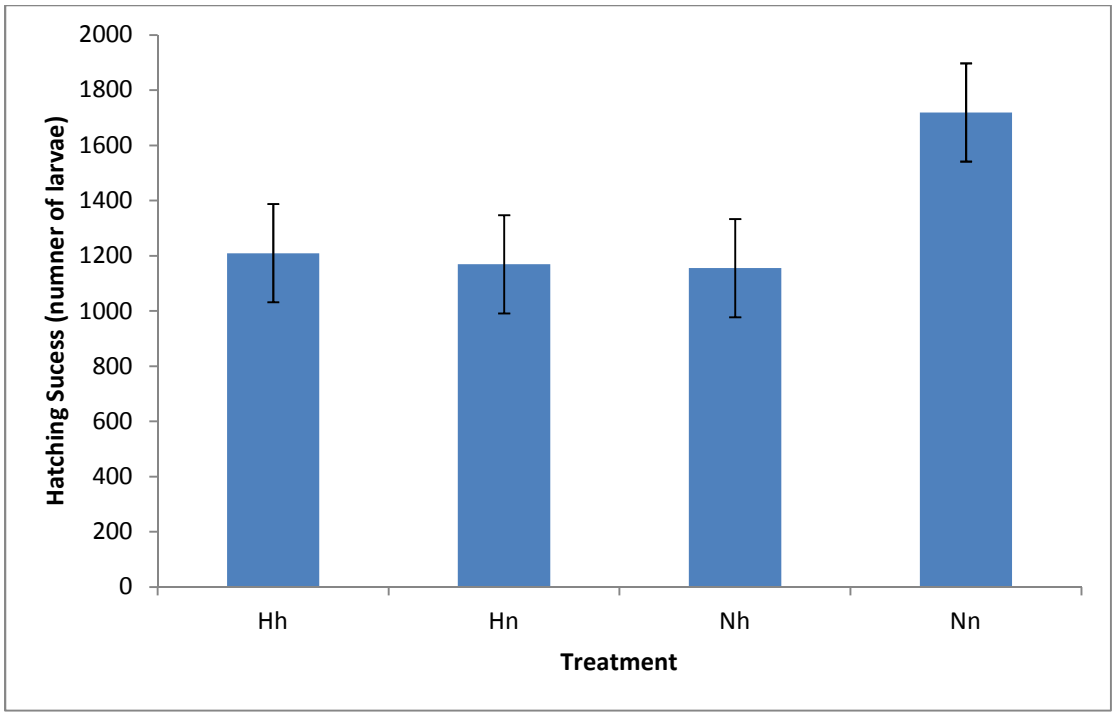


Figure 5: Hatching success of pacific oyster (*C. gigas*) fertilized eggs after one week of conditioning adult oysters followed by 24 hours of larval exposure. The bars represent the number of fertilized eggs out of an original stocking density of 2000 fertilized eggs hatched into larvae. Error bars are the standard error.

Based on these qualitative inspections, it was found that treatment Nn had significantly more normal larvae than the other three treatments that had low numbers of normal larvae (by adult exposure: $F_{1,16} = 62.44$, $p < 0.0001$; by larval exposure: $F_{1,16} = 22.2$, $p = 0.0002$; Table 3 and

Figure 6). It was also found that the two factors in the ANOVA, adult exposure and larval exposure, had a significant interaction ($F_{1,16} = 12.16$, $p = 0.003$, Figure 7). Additionally, according to a Tukey test, the percent that developed normally in the treatment Hh was not statistically different from the percent that developed normally in Hn ($t_{1,16} = -0.87$, $p = 0.8221$). Also, the percent that developed normally in the treatment Hn did not differ significantly from the percent that developed normally in the treatment Nh ($t_{1,16} = -2.26$, $p = 0.1507$).

Table 3: Table showing the ANOVA results for the percent of normally developing D-hinge larvae

Factor	Numerator Degrees of Freedom	Denominator Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Adult	1	16	1.172	1.172	62.44	<0.0001
Larval	1	16	0.417	0.417	22.2	0.0002
Interaction	1	16	0.228	0.228	12.16	0.003
Residual	16		0.300	0.019		

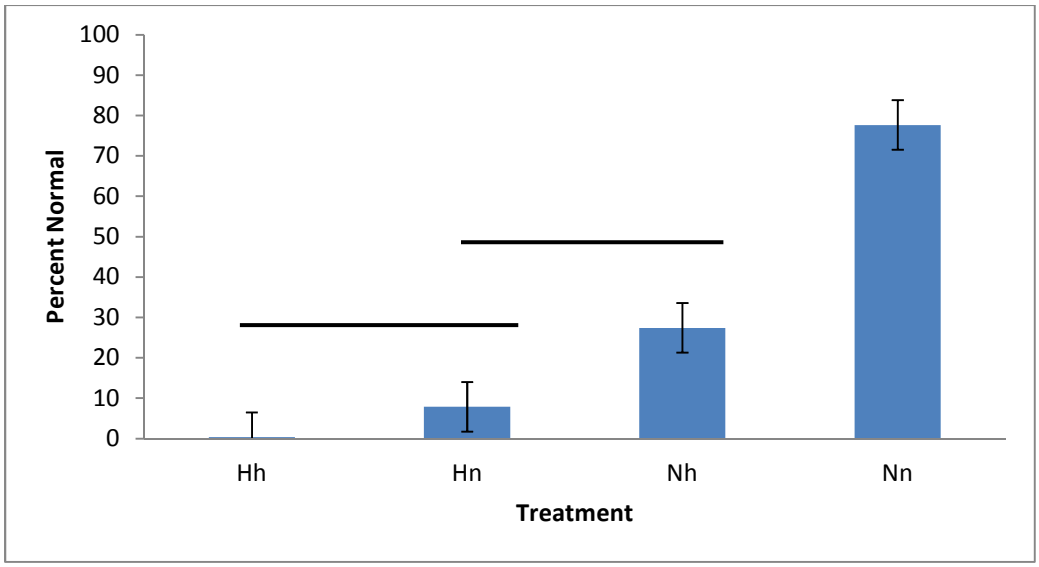


Figure 6: Average percent normal *C. gigas* larvae per sample in each of the four treatments. "Normal" larvae are defined as having a straight hinge and a clear D-shaped shell. Normal refers only to the shell shape, not to the development of tissue or internal organs. Error bars are the standared error. The lines running horizontal over the bars shows treatments did not differ significantly based on a Tukey test.

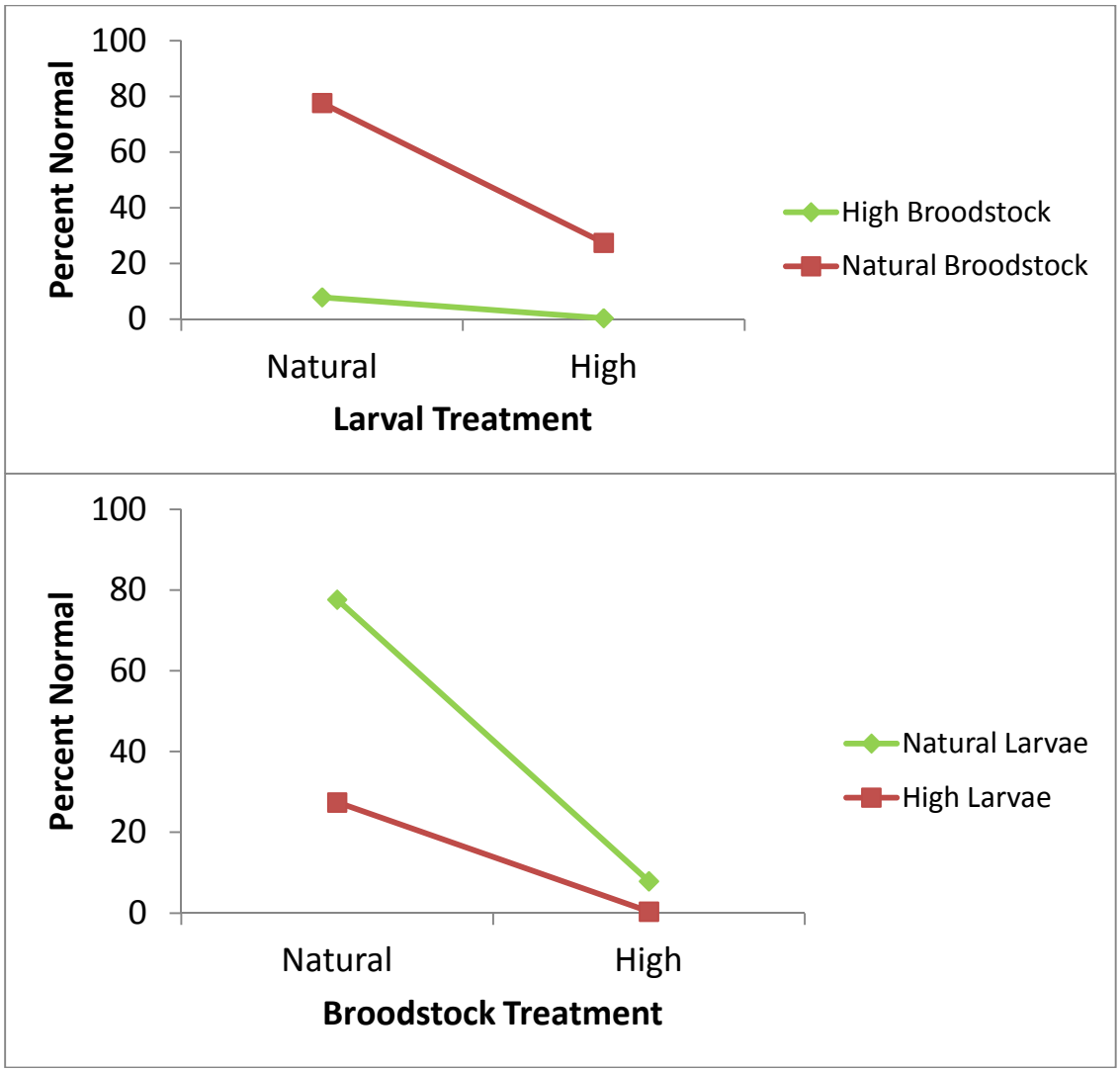


Figure 7: Interaction effects of 24-hour larval exposure and week-long adult exposure on the percent of normally developing larvae of Pacific oysters (*C. gigas*). "High broodstock" refers the oysters that were conditoned for one week in high Pco_2 water. "Low broodstock" refers the oysters that were conditoned for one week in low Pco_2 water. "High Larvae" refers to larvae reared for 24 hours in elevated Pco_2 water. "Low Larvae" refers to larvae reared from 24 hours in low Pco_2 water. These graphs suggest there was an additive affect of adult and larval exposure on the percent of larvae that develop noremally because the slopes of these lines go in the same direction with one line steeper than the other. This also shows that either that the adult exposure was more signifcant. On the other hand, the effects of the two factors could be more similar than they appear on this graph. This graph could look like this because the larvae from the broodstock condtioned in the high Pco_2 water started out at a low percentage of percent normal and can only decrease to zero percent normal, so the slope appears less steep.

Larval size

There was a significant difference in the shell lengths of larvae due only to adult exposure ($F_{1,15}$ = 64.44, p < 0.0001; by larval exposure: $F_{1,15}$ =0.00, p = 0.9650; interaction: $F_{1,15}$ = 1.03, p =0.3252; Table 4 and Figure 9). The larvae developed from broodstock conditioned in low Pco_2 water had larger shell lengths than the larvae that developed from the broodstock conditioned in high Pco_2 water. Similar to shell length, the shell height of the larvae was also only significantly affected by adult exposure to high Pco_2 water ($F_{1,15}$ = 127.72, p < 0.0001; by larval exposure: $F_{1,15}$ = 0.57, p =0.4625; interaction: $F_{1,15}$ = 8.78, p =0.0097; Table 5 and Figure 10). The larvae developed from broodstock conditioned in low Pco_2 water had larger shell heights than the larvae that developed from the broodstock conditioned in high Pco_2 water.

Table 4: Table showing the ANOVA results for the shell length

Factor	Numerator Degrees of Freedom	Denominator Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Adult	1	15	306.799	306.799	64.44	<0.0001
Larval	1	15	0.0095	0.0095	0.00	0.9650
Interaction	1	15	4.926	4.926	1.03	0.3252
Residual	15		71.412	4.761		

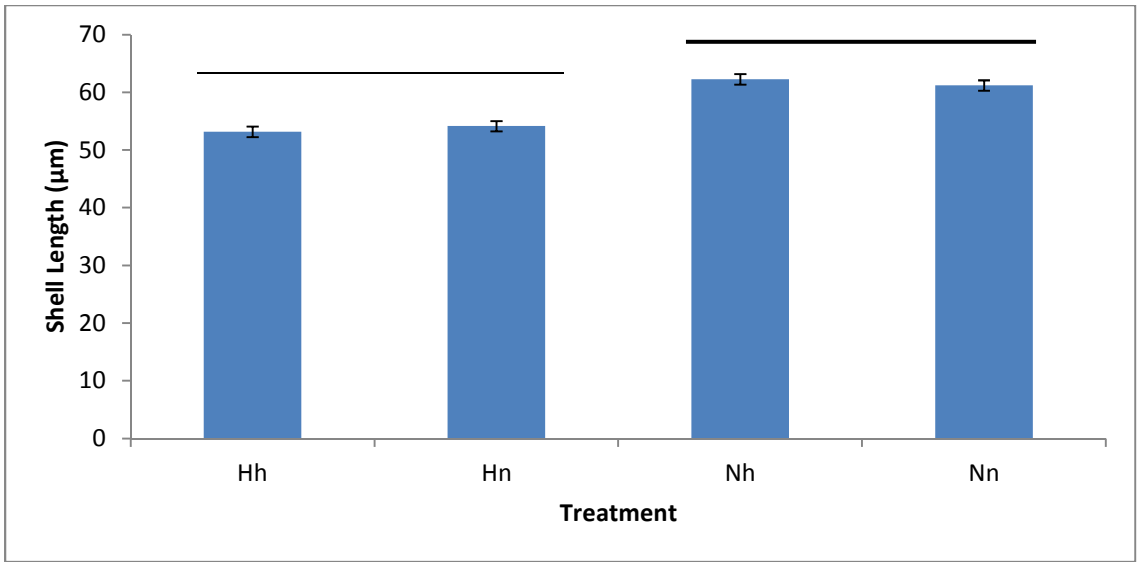


Figure 8: Average length of 24-hour old *C. gigas* D-hinge larvae from four treatment groups. The two letter codes (Hh, Hn, Nh, and Nn) represent the treatment names. Error bars are the standared error. The lines running horizontal over the bars shows treatments that did not differ significantly based on a Tukey test.

Table 5: Table showing the ANOVA results for the shell height

Factor	Numerator Degrees of Freedom	Denominator Degrees of Freedom	Sum of Squares	Mean Square	F value	p value
Adult	1	15	520.644	520.644	127.72	<0.0001
Larval	1	15	2.318	2.318	0.57	0.4625
Interaction	1	15	35.795	35.795	8.78	0.0097
Residual	15		61.148	4.077		

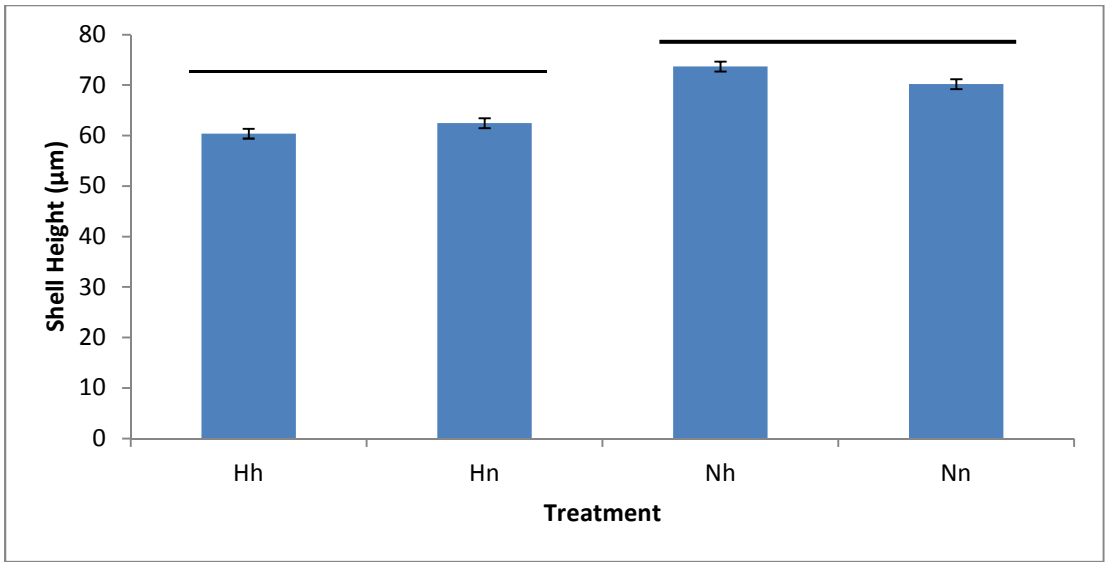


Figure 9: Average shell height of 24-hour old *C. gigas* D-hinge larvae from four treatment groups. The two letter codes (Hh, Hn, Nh, and Nn) represent the treatment names. Error bars are the standared error. The lines running horizontal over the bars shows treatments that did not differ significantly based on a Tukey test.

DISCUSSION

Many recent studies have focused on the effects of elevated Pco_2 on the larval stage of bivalves as well as the connections between life history stages (including: *C. gigas,* Kurihara et al. 2007; review, Kroeker 2010; *S. glomerata*, Parker et al. 2012; *C. gigas*, Barton et al. 2012; *O. lurida*, Hettinger et al. 2012, review*,* Gazeau et al. 2013; review, Parker et al. 2013). There have been far fewer studies that focus on the egg size as a possible connection between life history stages, especially in the Pacific oyster. This study found that eggs harvested from Pacific oysters conditioned in elevated Pco_2 water were smaller than eggs harvested from Pacific oysters conditioned in lower Pco_2 water (Figure 4). For many marine species, it has been well documented that the environment in which the mother lives can affect the size of the eggs that she produces (*B. neritina,* Allen et al. 2007; *M. californianus,* Phillips 2007; *N. pulcher,* Mileva et al. 2010; *Crassostrea* spp.*,* Powell et al. 2011). In adverse conditions, egg size decreases significantly because the mother must invest more energy to maintain her own fitness (*B. neritina,* Allen et al. 2007; *N. pulcher,* Mileva et al. 2010). There is some speculation that during suboptimal conditions, like ocean acidification, increased temperature, low food availability, or a combination of these factors, the energetic cost of maintaining tissues increases, which might cause organisms to undergo metabolic depression to prolong their survival by conserving energy (*C. gigas*, Lannig et al 2010). Some studies hypothesize that organisms go through an energetic "tradeoff", increasing the energy input in one life aspect, such as the organisms' ion or acid-base balance, at the cost of another like immune response or reproduction (*C. gigas*, Lannig et al 2010; review, Gazeau et al.; review, Parker et al. 2013). Based on previous studies on bivalve species spanning a similar amount of time as this experiment, the elevated ocean acidification conditions in which the broodstock from the "high" tank were conditioned were acidic enough to cause decreased calcification rates, decreased shell growth, or changes in immune responses (*C. virginica,* Waldbusser et al. 2011; *S. glomerata,* Parker et al. 2011; *C. nobilis* and *M.*

galloprovincialis, Matozzo et al. 2012; review, Parker et al. 2013). Over longer periods of time at the elevated level of acidification used in this study, some organisms developed a decreased immune response (*M. edulis,* Bibby et al. 2008), a decreased respiration rate (*R. decussatus,*Seibel and Walsh 2003), and higher levels of shell dissolution and oxidative stress (*C. virginica,* Tomanek et al. 2011). Therefore, it is valid to make the assumption that the eggs harvested from the broodstock conditioned in elevated Pco_2 were smaller than eggs harvested from broodstock conditioned in lower Pco_2 due to the stress that the broodstock faced during the conditioning period.

In this study, there were approximately 500 to 600 more hatched in treatment Nn, which had no stage exposed to the high Pco_2 water, than any of the three other treatments that had one or both stages exposed to high Pco_2 water (Figure 5). While Nn did not differ statistically from the other treatments, the increased hatching success in the treatment Nn is a strong trend in the data. In bivalve populations, less than 10% of larvae survive until recruitment (Gosselin and Qian 1997). With small success numbers like this, having 500 to 600 more fertilized eggs hatching to the larval stage could lead to significantly more larvae and breeding oysters in the future, which has important economic and population implications. Furthermore, 500 larvae represent 25% of the total 2000 fertilized eggs originally stocked in the treatment bottle. Thus, this is a fairly large difference across treatments based on the total number of larvae per treatment bottle. Therefore, the lack of statistical significance could be due to the high level of variability in the data.

This study also found that shell development and size were lower in the larvae that were developed from the oysters conditioned in elevated Pco_2 conditions (Figures 6, 9, and 10). This is consistent with previous studies that suggest elevated Pco_2 negatively affects the larval stage of *C. gigas* (Kurihara et al. 2007; Parker et al. 2010; Barton et al. 2012; Timmins-Schiffman et al. 2012; review, Parker et al. 2013). However, there was not a significant difference in the larval size or development between the two larval treatments (Nn vs. Nh, and Hn vs. Hh) within the two broodstock groups (high vs. low) (Figures 9 and 10). This contradicts previous studies that found

a difference in larval development based on the Pco_2 level of the water in which the larvae were reared (Parker et al. 2012; Barton et al. 2012). Furthermore, Parker et al (2012) found that the larvae developed from oysters conditioned in elevated Pco_2 performed better in the elevated Pco_2 water than the larvae developed from oysters conditioned in the lower Pco_2, which contradicts the result of the study presented in this paper. The different results could have occurred for multiple reasons. First, these two studies used different test species and Parker et al. (2012), states the responses to ocean acidification by different species of oysters, or within different populations of the same species, differed slightly from one another. During this study, though the Pco_2 levels in the larval treatments Nh vs. Nn and Hn vs. Hh were different at the beginning of the larval period, the Pco_2 levels did not differ significant from each other by the end of the larval rearing period. This change in the water chemistry could be a reason why the shell sizes were similar between the treatments Nh vs Nn and Hn vs Hh.

However, because the larval rearing conditions were similar by the end of the larval rearing period, and the larvae from the broodstock conditioned in low Pco_2 water performed better the larvae from the broodstock conditioned in the high Pco_2 water, it can be inferred that the egg quality (which was assumed based on size which differed significantly between broodstock treatment groups) significantly affected larval performance. Therefore, it appears that the effects of ocean acidification on one life history stage can be observed in later life history stages in the pacific oyster. This result supports previous ocean acidification studies on oyster species (*S. glomerata*, Parker et al. 2012; *O. lurida*, Hettinger et al. 2012). Some studies suggest there is only a small correlation between egg size and initial larval size (*M. californianus,* Phillips 2007). There are many other studies documenting the effects of decreased egg size on other life history aspects, like fertilization success and development time, that could affect larval size (Levitan 2000, 2006; Marshal and Bolton 2007; Havenhand et al. 2008). Hence, egg size is a likely connection between adults and offspring that can carry over the negative effects of ocean acidification.

An interesting next step would be to repeat this study with better control of the Pco_2 levels in the larval rearing environments to see how larval exposure affects the shell size. It would also be interesting to vary the conditioning time for the broodstock to investigate how the length of exposure to acidified water affects egg sizes and subsequent larval sizes. Finally, it would be interesting to extend the length of the larval exposure and growth time to see if these effects are carried over into later life history stages, or even later generations.

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