LARVAL RESPONSE TO PARENTAL LOW pH EXPOSURE IN THE PACIFIC OYSTER

CRASSOSTREA GIGAS

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ABSTRACT As negative effects of ocean acidification are experienced by coastal ecosystems, there is a growing trend to investigate the effect ocean acidification has on multiple generations. Parental exposure to ocean acidification has been shown to induce larval carryover effects, but whether acute exposure to a stressor as an adult can influence the larval generation long after the stress has been removed has yet to be tested. To assess how a temporary exposure to experimental ocean acidification affects the ecologically and commercially relevant Pacific oyster Crassostrea gigas, adult oysters were exposed to either low pH (7.31 ± 0.02) or ambient pH (7.82 ± 0.02) conditions for 7 wk. Oysters were then held for 8 wk in ambient conditions, and subsequently reproductively conditioned for 4 wk at ambient pH. After conditioning, the oysters were strip-spawned to create four families based on maternal and paternal ocean acidification exposure. The number of D-hinge larvae was counted 18 h postfertilization. A sex-specific brood stock response was observed, where female exposure to low pH conditions resulted in fewer D-hinge larvae. This study demonstrates that the effects of ocean acidification can last beyond the time from when the environmental perturbation is experienced. Broadening the understanding of environmental memory will be valuable when considering organismal ability to persist in the face of environmental change.

KEY WORDS: ocean acidification, maternal effect, carryover effect, Pacific oyster, Crassostrea gigas, D-hinge larvae, response timing

INTRODUCTION

Determining how parental exposure to ocean acidification carries over into early larval stages is important for understanding cumulative effects of climate-related environmental change. Gametogenesis is a key period during which parental exposure to ocean acidification can influence offspring (Donelson et al. 2018). Several studies exposing the Sydney rock oyster Saccostrea glomerata to high pCO2 conditions (856 μatm, pHNBS 7.89–7.90) during reproductive conditioning identified positive larval carryover effects (Parker et al. 2012, 2015, 2017). Specifically, larvae from parents exposed to low pH conditions were larger and developed faster in acidified conditions than those from parents reared in ambient pH conditions (Parker et al. 2012, 2015, 2017). Conversely, similar experiments conducted with the northern quahog Mercenaria mercenaria (also called hard clam) and the bay scallop Argopecten irradians demonstrated negative larval carryover effects (Griffith & Gobler 2017). Larvae from adult A. irradians and M. mercenaria exposed to low pH were more sensitive to acidified conditions than those spawned from parents exposed to ambient pH during reproductive conditioning (Griffith & Gobler 2017). These studies demonstrate the importance of parental exposure during reproductive conditioning (late-stage gametogenesis) on offspring.

As the Pacific oyster Crassostrea gigas (Thunberg, 1793) is a commercially and ecologically relevant species in much of the world, several research efforts have identified consequences of ocean acidification for distinct C. gigas life stages. Although fertilization still occurs under near-future ocean acidification conditions (Kurihara et al. 2007, Havenhand & Schlegel 2009, Boulais et al. 2018), fertilization success in acidified conditions is limited evidence, however, of how ocean acidification influences Pacific oysters across multiple generations.

The current study is the first to discern how exposure to experimental ocean acidification before reproductive conditioning affects larval abundance in Crassostrea gigas. This experiment not only describes how isolated exposure to low pH during early gametogenesis influences larvae but also provides information on the effects of acute pH exposure on adult gonad morphology. In addition, the study demonstrates how environmental perturbation experienced before reproductive maturity affects the subsequent generation, even if the stressor is long-removed.

MATERIALS AND METHODS

Experimental Overview

Experimental trials were conducted at the Kenneth K. Chew Center for Shellfish Research and Restoration at the National Oceanic and Atmospheric Administration Manchester Field
Station (47° 34’ 09.1” N 122° 33’ 19.0” W, Manchester, WA) in 2017. Adult hatchery-raised *Crassostrea gigas* (average shell length = 117.46 ± 19.16 cm) were acclimated in the facility for 10 days and then exposed to either low or ambient pH conditions for 48 days (Fig. 1). After pH exposure, oysters were held at ambient pH and water temperature conditions for 90 days. Oysters underwent reproductive conditioning for 22 days and were then strip-spawned. D-hinge larvae were counted 18 h after fertilization occurred.

**Experimental pH Exposure**

The experimental system consisted of a 1,610-L storage tank that fed two 757-L header tanks. Water from Clam Bay, WA, was pumped through a sand filter and then UV-treated. The UV-treated water passed through a set of three sock filters (100, 50, and 25 µm) and a degassing column. Once degassed, water passed through three more sock filters (25, then 10, and 5 µm) before entering the storage tank. The storage tank was outfitted with an off-gas vent and pump to recirculate water such that CO2 in the water could be equilibrated with atmospheric CO2. Equilibrated water flowed into the two header tanks, each of which fed three 50-L flow-through (1.2 L/min) experimental tanks (six experimental tanks in total). For all header and experimental tanks, pH in header and experimental tanks was continuously monitored using Durafet pH probes (Honeywell Toledo) to determine alkalinity. Salinity from discrete samples days 5, 33, and 48 were run on a T5 Excellence titrator (Mettler Toledo), and temperature (°C) was measured using a Traceable digital thermometer (Model 15-077, Fisher). To calibrate the pH probe, Tris buffer (0.08 M, 28.0 salinity) was prepared using 0.3603 mol of NaCl (J. T. Baker), 0.0106 mol of KCl (Fisher Scientific), 0.0293 mol MgSO4-(H2O)7 (Fisher Scientific), 0.0107 mol of CaCl2-2(H2O) (MP Biomedicals), 0.0401 HCl (J. T. Baker), and 0.0799 mol of Tris base (Fisher Scientific). Deionized water was added for a final volume of 1 L. Salinity, temperature, and pH measurements for the Tris buffer were obtained at five temperatures before measuring samples to generate a standard curve. This standard curve was used to calibrate the pH electrode and convert measured millivolts to pH units.

**Seawater Chemistry Analysis**

Twice a week, water samples (1 L) were collected from each header and oyster experimental tank. For each sample, salinity was measured with a bench/portable conductivity meter (Model 23226-505, VWR), pH (mV) was measured with a combination pH electrode (Model 11278-220, Mettler Toledo), and temperature (°C) was measured using a Traceable digital thermometer (Model 15-077, Fisher). To calibrate the pH probe, Tris buffer (0.08 M, 28.0 salinity) was prepared using 0.3603 mol of NaCl (J. T. Baker), 0.0106 mol of KCl (Fisher Scientific), 0.0293 mol MgSO4-(H2O)7 (Fisher Scientific), 0.0107 mol of CaCl2-2(H2O) (MP Biomedicals), 0.0401 HCl (J. T. Baker), and 0.0799 mol of Tris base (Fisher Scientific). Deionized water was added for a final volume of 1 L. Salinity, temperature, and pH measurements for the Tris buffer were obtained at five temperatures before measuring samples to generate a standard curve. This standard curve was used to calibrate the pH electrode and convert measured millivolts to pH units.

For total alkalinity measurements, duplicate seawater samples (250 mL) were collected from experimental tanks twice weekly and dosed with mercuric chloride (50 µL of 0.18 M solution) to preserve samples (Bandstra et al. 2006). Samples from days 5, 33, and 48 were run on a T5 Excellence titrator (Mettler Toledo) to determine alkalinity. Salinity from discrete samples was used to calculate total alkalinity, using the seawarb library in R (Gattuso et al. 2018). Calculated pH, total alkalinity,

![Figure 1](https://bioone.org/journals/Journal-of-Shellfish-Research)
temperature, and salinity were also used in seawater to calculate in situ pH, pCO₂, dissolved inorganic carbon (DIC), calcite saturation (Ωcalcite), and aragonite saturation (Ωaragonite) for days 5, 33, and 48. R code used to calculate water chemistry parameters is available (Venkataraman et al. 2018).

**Histological Analysis**

Twenty randomly selected *Crassostrea gigas* were lethally sampled before pH exposure for histological analyses. On the last day of low pH exposure, 10 oysters from each treatment—randomly selected from each tank—were also lethally sampled to assess gonadal status. For each sampled oyster, a piece of gonad tissue was cut and placed in a histology cassette. Gonad tissue in cassettes was fixed for histology using PAXgene Tissue Fix and Stabilizer and sent to Diagnostic Pathology Medical Group, Inc. (Sacramento, CA) for staining with hematoxylin and eosin and slide preparation. Tissues exposed to ambient pH were confounded during processing, preventing any tank identification. Maturation state and organism sex was evaluated histologically at 40× magnification (Fabioux et al. 2005, Enríquez-Díaz et al. 2008).

**Reproductive Conditioning**

Following 7 weeks of low pH exposure, oysters were returned to a common garden and maintained at ambient pH conditions for 8 wk. Afterward, oysters were reproducibly conditioned. Water temperatures and food quantity are known to regulate the timing, speed, and intensity of gametogenesis in *Crassostrea gigas* (Enríquez-Díaz et al. 2008). Conditioning protocol was modeled after standard hatching practices (Molly Jackson, brood stock manager at Taylor Shellfish, personal communication, June 2017). Water temperature was raised from ambient conditions (13°C to 23°C over 3 wk (1°C/2 days) because optimal temperature for *C. gigas* gametogenesis is between 18°C and 26°C (Parker et al. 2010). Conditions were maintained at 23°C for one week before spawning. During conditioning, *C. gigas* were fed 700–800 mL of Shellfish Diet 1800 daily (Helm et al. 2004).

**Strip-Spawning and Larval Rearing**

After reproductive conditioning, all surviving oysters were prepared for strip-spawning. A sample of gonad from each individual was assessed for presence of active sperm or eggs using a microscope at 10× magnification. Only *Crassostrea gigas* with active sperm or eggs were used for crosses (n_male, low = 6, n_female, low = 22, n_male, ambient = 6, and n_female, ambient = 26). Presence of mature gametes and ripe oysters indicated that oysters were in good condition and not affected by use of Shellfish Diet 1800 instead of live algae during reproductive conditioning. For each treatment (low pH and ambient conditions), 1 g of mature gonad from each ripe female was pooled. The number of eggs in both the ambient and low pH pools was determined to determine the number of eggs used for parental crosses. Parental crosses were created using 210,000 eggs from the female egg pools and sperm (200 μL) from individual males.

Four half-sibling families were created based on parental pH exposure: low pH female (pool) × low pH male, low pH female (pool) × ambient pH male, ambient pH female (pool) × low pH male, and ambient pH (pool) female × ambient pH male. These crosses were conducted using pooled eggs from either low pH or ambient pH females and sperm from one of six males within each pH treatment (e.g., low pH female pool × low pH male-01, low pH female pool × low pH male-02, low pH female pool × low pH male-06), totaling 24 crosses. All crosses were performed in duplicate, resulting in 48 separate fertilization events.

Fertilization was carried out in plastic beakers (1 L) for 20 min with static 23°C filtered seawater (1 μm) in ambient pH conditions. After confirming polar body formation, beaker contents were transferred to larger plastic tanks (19 L) with aerated, static 23°C filtered seawater (1 μm) for 18 h of incubation. Duplicate containers were combined 18 h post-fertilization, and D-hinge larvae were counted for each cross (n = 24).

**Statistical Analyses**

Differences in in situ pH, total alkalinity, pCO₂, DIC, Ωcalcite, and Ωaragonite between pH treatments were evaluated with a one-way ANOVA. Because tissue samples were confounded during histological processing, a binomial GLM model was used to compare gonad maturation between pH treatments. Differences in sex ratios between pH treatments were evaluated using a chi-square test of homogeneity. To identify differences in D-hinge larval counts, a linear mixed model was used, with sire and female egg pool as random effects. Differences in D-hinge larval counts by female treatment were assessed using a similar linear mixed model, with only sire as a random effect. Normality of data, as well as independence and homoscedasticity, was verified visually. All statistical analyses were carried out in R (version 3.4.0). R scripts are available in the supplementary GitHub repository (Venkataraman et al. 2018).

**RESULTS**

**Water Chemistry**

Pacific oysters exposed to low pH experienced different water chemistry parameters than those in the ambient pH treatment (Table 1). Using water samples from days 5, 33, and 48, pH (one-way ANOVA; F₁,₁₆ = 5.838.7810, P = 6.1165e-22), pCO₂ (one-way ANOVA; F₁,₁₆ = 235.4018, P = 5.4421e-11), DIC (one-way ANOVA; F₁,₁₆ = 7.1222, P = 0.0168), Ωcalcite (one-way ANOVA; F₁,₁₆ = 528.9468, P = 1.0989e-13), and Ωaragonite (one-way ANOVA; F₁,₁₆ = 526.5207, P = 1.1389e-13) were significantly lower in the low pH treatment. Total alkalinity, however, was not significantly different between pH treatments (one-way ANOVA; F₁,₁₆ = F = 1.382, P = 0.2570).

**Gonad Maturation**

A binomial GLM was used to compare gonad maturation of individuals sampled before and immediately after pH exposure, but before reproductive conditioning. The most parsimonious model included only sampling time (before or after pH treatment). Gonad maturation status was not significantly different between *Crassostrea gigas* sampled before and after pH treatment (binomial GLM; F₁, 37 = 0.7973, P = 0.3442). In addition, maturation status was not different between pH treatments (binomial GLM; F₁, 36 = 2.2675, P = 0.1408). No sampled oysters possessed fully mature gametes, but some males sampled appeared to be undergoing resorption (Venkataraman et al. 2018). Sex ratios were also similar between low and
TABLE 1.

<table>
<thead>
<tr>
<th>Day</th>
<th>Control</th>
<th>Experiment</th>
<th>Control</th>
<th>Experiment</th>
</tr>
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<tbody>
<tr>
<td>pH</td>
<td>7.82 ± 0.004</td>
<td>7.33 ± 0.004</td>
<td>7.33 ± 0.004</td>
<td>7.33 ± 0.004</td>
</tr>
<tr>
<td>Total alkalinity (μmol/kg)</td>
<td>2,332.36</td>
<td>2,307.41</td>
<td>2,332.36</td>
<td>2,332.36</td>
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<tr>
<td>pCO2 (μatm)</td>
<td>7.34 ± 0.004</td>
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The seacarb library in R was used to calculate total alkalinity, and in situ pCO2, dissolved inorganic carbon (DIC), calcite saturation (Ωcalcite), and aragonite saturation (Ωaragonite) for each ocean acidification (OA) treatment. A linear mixed effect model (LMM) was used to analyze the data. For each OA treatment: 1.382, 0.0042, and 0.42 for 0.8530% and 3.1623% of total variance, respectively. Significant differences were observed with sire accounting for 0.3116053% of total variance.

DISCUSSION

The present study is the first to document the transgenerational influence of ocean acidification on Pacific oysters. Larval Crassostrea gigas were negatively impacted when maternal brood stock were exposed to low pH (pH = 7.31), suggesting a maternal carryover effect. The experimental design of this study is also unique—adult C. gigas experienced low pH conditions 3 mo before reproductive conditioning, and then were kept solely in ambient pH conditions through strip-spawning and larval rearing. Because environmental perturbation experienced before Pacific oysters were mature still affected larval oysters, the results indicate a role for environmental memory in C. gigas response to ocean acidification. Mechanisms for transgenerational environmental memory have been explored in response to acute stressors in other species. For example, Daphnia magna exposed to high salinity conditions had altered DNA methylation patterns, and these patterns were inherited by the following three nonexposed generations (Jeremias et al. 2018). Significant carryover effects observed in C. gigas—solely exposed to low pH when immature—broaden the current understanding of stressor timing and its effect on organismal physiology.

Although it is evident that acute exposure to low pH experienced by adult Crassostrea gigas resulted in detrimental effects for larvae, the fact that larvae were not reared in acidified conditions makes cross-study comparison difficult. If C. gigas larvae were also reared in acidified conditions, it is possible that larvae with a history of parental exposure to experimental ocean acidification may have exhibited a negative carryover effect on larval growth and performance. Negative carryover effects have been found in other marine invertebrate taxa, but all studies involved exposure to experimental ocean acidification during reproductive conditioning and larval rearing in acidified conditions. The tanner crab Chionoecetes lalandii (also known as clam) and the bay scallop Argopecten irradians developed slower when parents were reproductively conditioned in low pH conditions (pH = 7.4) (Griffith & Gobler 2017). In addition, larvae with a history of parental low pH exposure were more vulnerable to additional stressors such as thermal stress, limited food, and harmful algae exposure (Griffith & Gobler 2017). Although C.
transgenerational exposure exhibited faster development, but the present study. Sydney rock oyster larvae with a history of pCO2 treatment of 856 atm (pH \( \text{P}_{\text{H}} = 7.89–7.90 \)) during reproductive conditioning, the resultant larvae were larger and developed faster in acidified conditions than larvae from parents exposed to ambient conditions (Parker et al. 2012). This positive carryover effect was found to persist in the \( F_2 \) generation. Inacidified conditions, \( F_0 \) offspring with a history of transgenerational (\( F_0 \) and \( F_1 \)) pCO2 exposure grew faster and demonstrated fewer shell abnormalities (Parker et al. 2015). Although species-specific responses can certainly explain the observed differences in larval phenotypes, it is also likely that inconsistencies in treatment conditions between experiments resulted in dose-dependent effects. Parker et al. (2012, 2015, 2017) used a high pCO2 treatment of 856 \( \mu \text{atm} \) (pH \( = 7.89–7.90 \)), with a control of 380–385 \( \mu \text{atm} \) (pH \( = 8.19–8.20 \)). Therefore, the elevated pCO2 treatment used in Parker et al. (2012, 2015, 2017) is similar to the ambient pH treatment (7.82; pCO2 \( = 747.51–912.22 \)) in the present study. Sydney rock oyster larvae with a history of transgenerational exposure exhibited faster development, but exhibited similar survival and were only 10% larger in acidified conditions than larvae with no transgenerational exposure history (Parker et al. 2012). With a relatively smaller effect size and a milder treatment than that used in this study, it is possible these studies are not at odds but reflect dose-dependent effects on larval phenotypes. Negative carryover effects demonstrated in this study and in Griffith and Gobler (2017) can also be attributed to similar treatment pH levels (Griffith & Gobler 2017: pH \( \text{F} = 7.4; \text{this study: pH} = 7.31 \)). Both of these studies used treatment levels more extreme than the International Panel on Climate Change projections for open ocean acidification, but consistent with coastal and estuarine acidification scenarios experienced at study locations (Feely et al. 2010, Griffith & Gobler 2017, Pelletier et al. 2018). More research is required to understand how location-specific conditions will affect multiple generations in a single species.

Although the effect of water chemistry on gametogenesis has been recorded in other taxa, it is unlikely that a low pH exposure occurring 3 mo before reproductive conditioning could have affected gonad maturation. Studies in which reproductive conditioning and experimental ocean acidification occur concurrently have demonstrated negative effects on maturation and fecundity. Gametogenesis, especially oogenesis, was disrupted in the eastern oyster Crassostrea virginica that experienced severe ocean acidification conditions during reproductive conditioning (pH \( \text{F} = 7.71, 5.584 \mu \text{atm} \)) (Boulais et al. 2017). The green sea urchin Strongylocentrotus droebachiensis exposed to high pCO2 (1,200 \( \mu \text{atm} \)) conditions for 4 mo demonstrated low fecundity (Dupont et al. 2013), and Saccostrea glomerata conditioned in high pCO2 (856 \( \mu \text{atm} \)) conditions exhibited reduced rates of gametogenesis, smaller gonad area, and reduced fecundity (Parker et al. 2018). Gonad histology from Crassostrea gigas taken immediately after low or ambient pH exposure did not indicate any differences in maturation state, or interaction between sex and maturation state, between treatments. Even if fecundity or rates of gametogenesis differed between treatments, a return to ambient conditions for 3 mo may have reversed any detrimental effects.

Reduced Crassostrea gigas larval abundance could have been a result of altered maternal provisioning in female oysters exposed to low pH conditions. In the face of stressors, females can either increase maternal provisioning (Allen et al. 2008, Sunday et al. 2011)—diverting more resources, such as lipids or proteins, into eggs—or decrease provisioning because of energetic constraints (Liu et al. 2010, Uthicke et al. 2013). For example, changes in fatty acid provisioning from maternal exposure to high pCO2 conditions (2,300 \( \mu \text{atm} \)) in the Atlantic silverside Menidia menidia resulted in lower embryo survival when eggs lacked certain fatty acids (Snyder et al. 2018). This phenomenon, however, was not documented in the Sydney rock oyster; although elevated pCO2 conditions (856 \( \mu \text{atm} \)) reduced the amount of energy invested in maternal gonads, these conditions did not impact. Saccostrea glomerata egg size or total lipid content (Parker et al. 2018). Because adult C. gigas did not experience environmental perturbation after low pH exposure and received enough food to spawn well, any impact on maternal provisioning and subsequent larval abundance was likely a result of low pH 3 mo before reproductive conditioning.

The documented effect on Pacific oyster larval abundance 4 mo after low pH exposure indicates an important role for environmental memory in Crassostrea gigas response to ocean acidification.
acidiﬁcation. Low pH exposure may have induced epigenetic modifications (e.g., changes in DNA methylation) in adult C. gigas. Studies of ﬁsh and shellﬁsh aquaculture species have demonstrated environmentally induced epigenetic modiﬁcations that modify phenotypic responses in organisms (Gavery & Roberts 2017). One notable study on C. gigas examined parental effects of adult pollutant exposure on offspring (Rondon et al. 2017). Spat from parents exposed to the herbicide diuron had differential methylation in coding regions, with some changes leading to differential gene expression (Rondon et al. 2017). This research indicates that a mechanism crucial for phenotypic plasticity and acclimation across generations exists, and this knowledge can be analyzed in the context of climate-related environmental stressors. Epigenetic modiﬁcations in response to ocean acidiﬁcation have been documented in coral species (Putnam et al. 2016), but not in molluscs. Several experimental ocean acidiﬁcation studies, however, hint at the role of epigenetic memory. The Olympia oyster Ostrea lurida exposed to high pCO2 (1,000 μatm) conditions still grew less in the juvenile life stage than the counterparts reared in ambient pCO2, even after the stressor had been removed (Hettinger et al. 2013). Similarly, transgenerational acclimation of Saccostrea glomerata larvae with a history of exposure to acididiﬁed conditions could be explained by changes in epigenome that aﬀect organismal performance (Parker et al. 2012, 2015, 2017). Methylation levels are known to increase over the course of gametogenesis, with male and female C. gigas exhibiting signiﬁcantly diﬀerent methylation patterns (Zhang et al. 2018). If epigenetic modiﬁcations were acquired by female oysters during low pH exposure, it could explain why a signiﬁcant eﬀect on larval abundance was detected 4 mo after the exposure ended. Epigenetic mechanisms and altered maternal provisioning are not necessarily mutually exclusive—changes in the methylome could inﬂuence maternal provisioning—and both could contribute to the results observed in this study.

The results of this study emphasize the need to broaden the scope of when environmental perturbation experienced by an organism is considered stressful, and when an eﬀect can be detected. Although there was no observable eﬀect on adult gonad maturation right after low pH exposure, signiﬁcant diﬀerences in larval abundance were detected 4 mo after the exposure ended. Stressor timing and duration can impact transgenerational responses between mature parents and oﬀspring (Donelson et al. 2018). Although experimental ocean acidiﬁcation (pH 7.7; pCO2 = 800 μatm) increased female investment in amphipods (Gammarus locustae), the subsequent generation exhibited fewer eggs and lower fecundity in the same conditions (Borges et al. 2018). Transgenerational beneﬁts of maternal exposure to diﬀerent temperatures (17°C or 21°C) in the three-spined stickleback Gasterosteus aculeatus diﬀered based on exposure duration (Shama & Wegner 2014). Grandparents (F0) were only exposed to treatment temperatures during reproductive conditioning, whereas parents (F1) experienced either temperature over the course of development. The F1 generation exhibited temperature tolerances similar to the F0 maternal rearing environment, but the F2 generation tolerance was more similar to the F0 generation than the F1 generation (Shama & Wegner 2014). The present study demonstrates that length and timing of environmental perturbation experienced by immature individuals can still aﬀect offspring. Massamba-N’Siala et al. (2014) elucidated a similar phenomenon with the marine polychaete Ophryotrocha labronica: oﬀspring experienced positive carryover eﬀects of female exposure to temperature conditioning only when mothers were exposed to these conditions during late oogenesis; exposure during early oogenesis led to negative carryover eﬀects. More research should be conducted to understand how stressor timing, speciﬁcally before reproductive maturity, can impact carryover eﬀects.

Most other experiments investigating stressor timing are conducted in a multiple stressor framework (Gunderson et al. 2016). For example, elevated temperatures and low salinity had synergistic eﬀects on Ostrea lurida when they were co-occurring stressors, but 2–4 wk of recovery in between stressors negated these eﬀects (Bible et al. 2017). Incorporating recovery time in a single-stressor experimental design is also crucial for accurately understanding how environmental perturbation impacts organism physiology. Exposure at one point in time may elicit a response much later in time, in a diﬀerent environmental setting, or in a diﬀerent generation, as evidenced by the present study and Hettinger et al. (2013). The experimental design in the present study is unique, featuring a signiﬁcant recovery time between low pH exposure and spawning. More single-stressor experiments should incorporate lag times between exposure to stress and measuring response variables to understand if these responses change over time. Adding a multigenerational component to such experiments can elucidate if acute exposures generate carryover eﬀects.

Signiﬁcant decreases in larval abundance 4 mo after brood stock was exposed to acididiﬁed seawater have implications for both aquaculture and natural Crassostrea gigas populations. Parents and oﬀspring—or even diﬀerent oﬀspring life stages—may not experience the same environmental chemistry. For example, upwelling conditions aﬀecting adult C. gigas may subside once spawning occurs. Long-term monitoring of wild Paciﬁc oyster populations, with detailed environmental chemistry reporting, will be crucial for understanding how brief exposures to adverse conditions aﬀect reproductive success and larval abundance in the ﬁeld. Responses to stressors should not only be documented during and after the perturbation occurs but also for an extended time afterward. Hatchery-reared C. gigas larvae can also experience diﬀerent conditions than brood stock. Facilities unable to control water chemistry conditions may be exposing immature individuals to environmental perturbations that could aﬀect larvae once spawned. The success of “priming”—exposing C. gigas to stressful conditions to induce environmental memory and increase ﬁtness—hinges on the identiﬁcation of “programming windows” (Gavery & Roberts 2017). The present study shows that the period of time before reproductive conditioning can be important for transferring environmental memory, although only negative carryover eﬀects have been demonstrated in C. gigas.

CONCLUSION

Four months after adult Crassostrea gigas experienced experimental ocean acidiﬁcation, larval abundance of female oysters exposed to low pH was signiﬁcantly lower than those exposed to ambient pH 18 h postfertilization. Not only did this experiment elucidate intergenerational eﬀects of ocean acidiﬁcation on the Paciﬁc oyster but it also demonstrated a need to consider the timing of altered environmental conditions on organismal physiology. Although adult oysters experienced a
low pH stressor before reproductive conditioning, larval abundance was still significantly affected. Therefore, conditions experienced by aquaculture brood stock before reproductive conditioning should be taken into consideration. Likewise, these results should be considered when modeling large-scale ecosystem responses to ocean change. Future work on multigenerational responses to ocean acidification should investigate how exposure to adverse conditions while an organism is immature can affect reproductive success and offspring fitness. The significant lag time between the end of the low pH exposure and spawning possibly indicates some form of epigenetic “memory.” Additional research is needed to investigate the degree of environmental memory that can be maintained and the contributing epigenetic phenomenon.

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LITERATURE CITED


