



Carry-over effects of temperature and pCO₂ across multiple Olympia oyster populations

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Abstract:	Impacts of adult exposure to elevated winter temperature and altered carbonate chemistry on reproduction and offspring viability were examined in the Olympia oyster (<i>Ostrea lurida</i>). Three distinct populations of adult, hatchery-reared <i>O. lurida</i> , plus an additional cohort spawned from one of the populations, were sequentially exposed to elevated temperature (+4°C, at 10°C), followed by elevated pCO ₂ (+2204 µatm, at 3045 µatm) during winter months. Male gametes were more developed after elevated temperature exposure and less developed after high pCO ₂ exposure, but there was no impact on female gametes or sex ratios. Oysters previously exposed to elevated winter temperature released larvae earlier, regardless of pCO ₂ exposure. Those exposed to elevated winter temperature as a sole treatment produced more larvae

	<p>per day, but when oysters were also exposed to high pCO₂ there was no effect. These combined results indicate that elevated winter temperature accelerates <i>O. lurida</i> spermatogenesis, resulting in earlier larval release and increased production, with elevated pCO₂ exposure negating effects of elevated temperature. Offspring were reared in common conditions for one year, then deployed in four bays for three months. Offspring of parents exposed to elevated pCO₂ had higher survival rates in two of the four bays, which had distinct environmental conditions. This carryover effect demonstrates that parental conditions can have substantial ecologically relevant impacts that should be considered when predicting impacts of environmental change.</p>



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Dear Dr. Schimel,

We are pleased to submit the enclosed manuscript, “Carry-over effects of temperature and pCO₂ across multiple Olympia oyster populations,” for consideration in Ecological Applications.

Recent studies have considerably shifted our understanding of heritability, showing that ancestral exposures to environmental conditions can be transferred to future generations. For marine species, these carryover effects must now be considered to predict ecological changes due to warming and acidifying.

Our novel study shows that winter conditions substantially alter bivalve phenology and offspring physiology. We detected carryover effects of parental pCO₂ exposure in the Olympia oyster, *Ostrea lurida*, that depended upon environmental conditions. We also report that winter warming will result in precocious spawning and more frequent larval production in the spring, but acidification during the same season will counter effects of warming. Additionally, this study has four unique aspects which enhance findings:

- 1) Adults oysters were exposed to varying CO₂ during winter conditions, rather than during reproductive development and spawning, as is typical of carryover effect studies to date. Winter exposure to biologically challenging carbonate chemistry is more relevant than other seasons. We show that winter exposure matters.
- 2) Reproductive response was measured by volitionally spawning oysters, rather than artificially quantifying or stripping gonad. We provide results relevant to both wild oysters struggling to rebound, and the aquaculture industry which relies on effectively predicting broodstock reproductive cycles to collect larvae.
- 3) Offspring were reared in common conditions for one year prior to testing in the field, rather than reared under treatments. We show that carryover effects of parental environment persist, and impact whether a juvenile will survive in the stressors of the intertidal.

4) We captured intra-species variation by testing four groups of adult *O. lurida* with known, shared environmental histories, but bred from phenotypically distinct populations.

This is the first study to assess reproductive impacts of ocean acidification and warming, and to detect intergenerational carryover effects in the flat oysters, *Ostrea* spp. Our findings inform the restoration of wild flat oysters, which are distributed globally. Novel observations of intergenerational carryover effects in *Ostrea* also augment findings in other marine taxa to support the broader theory of beneficial transgenerational plasticity. Now, parental winter environment prior to spawning must be considered in biological response studies and ecosystem predictions.

This manuscript is not under consideration by another journal, and all authors approve of the manuscript and its submission to Ecological Applications.

Thank you for your consideration.

Sincerely,
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1 **Title:** *Carry-over effects of temperature and $p\text{CO}_2$ across multiple *Olympia* oyster populations*

2 **Running Title:** *Carry-over effects in the *Olympia* oyster*

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12 Abstract

13 Impacts of adult exposure to elevated winter temperature and altered carbonate chemistry
14 on reproduction and offspring viability were examined in the Olympia oyster (*Ostrea lurida*).
15 Three distinct populations of adult, hatchery-reared *O. lurida*, plus an additional cohort spawned
16 from one of the populations, were sequentially exposed to elevated temperature (+4°C, at 10°C),
17 followed by elevated pCO₂ (+2204 µatm, at 3045 µatm) during winter months. Male gametes
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19 exposure, but there was no impact on female gametes or sex ratios. Oysters previously exposed
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26 in four bays for three months. Offspring of parents exposed to elevated pCO₂ had higher survival
27 rates in two of the four bays, which had distinct environmental conditions. This carryover effect
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29 should be considered when predicting impacts of environmental change.

30 **Keywords:** *Ostrea*, acidification, pH, reproduction, winter, phenology, intergenerational,
31 transgenerational, climate change

Introduction

The repercussions of ocean warming and acidification on marine invertebrate physiology are complex, but significant recent advances indicate that early life stages of calcifying taxa are particularly vulnerable (Byrne & Przeslawski, 2013; Kurihara, 2008; Przeslawski, Byrne, & Mellin, 2015). More recently, the focus has shifted to whether early stages benefit from ancestral exposures, based on evidence that memory of environmental stressors can be transferred between generations (Diaz, Lardies, Tapia, Tarifeño, & Vargas, 2018; Kong *et al.*, 2019; Massamba-N'Siala, Prevedelli, & Simonini, 2014; Putnam & Gates, 2015; Ross, Parker, & Byrne, 2016). Beneficial, or positive, carryover effects may be important acclimatory mechanisms for marine invertebrates, particularly those that evolved in dynamic environments, such as estuaries and the intertidal (Donelson, Salinas, Munday, & Shama, 2018; Gavery & Roberts, 2014). These carryover effects are defined as transgenerational when they persist in generations that were never directly exposed. Intergenerational, or parental, effects may be due to direct exposure as germ cells (Perez & Lehner, 2019). A foundational series of studies on the Sydney rock oyster (*Saccostrea glomerata*) provide strong evidence for intergenerational carryover effects in estuarine bivalves. Adult *S. glomerata* exposed to high pCO₂ produced larger larvae that were less sensitive to high pCO₂, and the effect persisted in the successive generation (Parker *et al.*, 2012, 2015). In the presence of secondary stressors, however, parental high pCO₂ exposure rendered larvae more sensitive (Parker *et al.*, 2017). Intergenerational carryover effects are increasingly documented in larvae across other bivalve species, and are beneficial in the mussels *Mytilus chilensis* (Diaz *et al.*, 2018) and *Mytilus edulis* (but not juveniles) (Kong *et al.*, 2019; Thomsen *et al.*, 2017), and detrimental in the clam *Mercenaria mercenaria*, the scallop

Argopecten irradians (Griffith & Gobler, 2017), and the oyster *Crassostrea gigas* (Venkataraman, Spencer, & Roberts, 2019).

Preliminary intergenerational studies in bivalves are promising, but the body of work is still narrow in scope. Nearly all studies have exposed parents to stressors during denovo gamete formation (gametogenesis). For many temperate bivalve species, this occurs seasonally in the spring (Bayne, 1976). Yet, challenging periods of acidification and warming can occur during other times of the year (Evans, Hales, & Strutton, 2013; Joesoef, Huang, Gao, & Cai, 2015; McGrath, McGovern, Gregory, & Cave, 2019). The most corrosive carbonate environment in the Puget Sound estuary in Washington State, for example, commonly occurs in the winter when many species are reproductively inactive, while favorable conditions are in the spring when gametogenesis coincides with phytoplankton blooms (Pelletier, Roberts, Keyzers, & Alin, 2018). Thus, adult exposure to severely corrosive conditions during gametogenesis may not represent the natural estuarine system. To our knowledge, one study has assessed carryover effects of pre-gametogenic acidification in a bivalve, the oyster *C. gigas*, and found negative maternal carryover effects on larval survival (Venkataraman *et al.*, 2019), indicating that pre-gametogenic exposure also matters. No studies have yet attempted to examine intergenerational carryover effects of combined winter acidification and warming in bivalves.

To best predict whether intergenerational carryover effects will be beneficial or detrimental, it is also crucial to understand how warming and acidification will impact fertility and reproductive phenology. Temperature is a major driver of bivalve reproduction, and modulates gametogenesis (Joyce, Holthuis, Charrier, & Lindegarth, 2013; Maneiro, Pérez-Parallé, Pazos, Silva, & Sánchez, 2016; Oates, 2013), influences sex determination (Santerre *et al.*, 2013) and, in many species, triggers spawning (Fabioux, Huvet, Le Souchu, Le Pennec, &

Pouvreau, 2005) (alongside other factors such as photoperiod, nutrition, lunar/tidal phases). Year-round warming may result in unexpected impacts to larval competency resulting from changes to reproduction. For instance, some temperate bivalve species have a thermal threshold for gametogenesis and enter a period of reproductive inactivity, or “quiescence”, which is believed necessary for successive spawning (Giese, 1959; Hopkins, 1937; Loosanoff, 1942). Warmer winters brought on by global climate change (IPCC, 2013) may therefore shift species’ reproductive cycles to begin earlier, or eliminate seasonality altogether, resulting in poorly provisioned or ill-timed larvae (Chevillot *et al.*, 2017). Such impacts were clearly demonstrated using a long-term dataset (1973-2001) of estuarine clam *Macoma balthica* reproduction and temperature. Mild winters and earlier springs resulted in low fecundity, earlier spawning, and poor recruitment, which was largely explained by a phenological mismatch between spawning and peak phytoplankton blooms (Philippart *et al.*, 2003). The impacts of winter acidification on estuarine bivalve reproduction are less predictable. The few studies to date show that high pCO₂ delays gametogenesis in the oysters *S. glomerata* and *Crassostrea virginica* (Boulais *et al.*, 2017; Parker *et al.*, 2018), but both studies exposed oysters during gametogenesis. Acidification during the winter months could increase energetic requirements (Sokolova, Frederich, Bagwe, Lannig, & Sukhotin, 2012), and deplete glycogen reserves that are later utilized for gametogenesis in the spring (Mathieu & Lubet, 1993), but this hypothesis has yet to be tested.

The purpose of this study was to assess whether warmer, more acidic winters will affect fecundity and offspring viability in the Olympia oyster, *Ostrea lurida*. The Olympia is the only oyster species native to the Pacific coast of North America (McGraw, 2009). Overharvest and pollution devastated populations in the early 1900’s, and today 2-5% of historic beds remain (Blake & Bradbury, 2012; Polson & Zacherl, 2009). Restoration efforts are afoot, but *O. lurida*

may be further challenged by changing conditions, which are amplified along the Pacific coast (Barton, Hales, Waldbusser, Langdon, & Feely, 2012; Feely, Klinger, Newton, & Chadsey, 2012; Feely, Sabine, Hernandez-Ayon, Ianson, & Hales, 2008). Like other invertebrate species (Kelly, Padilla-Gamiño, & Hofmann, 2013; Parker, Ross, & O'Connor, 2011; Sanford & Kelly, 2011; Sunday *et al.*, 2014; Thompson, O'Connor, Parker, Ross, & Raftos, 2015), *O. lurida* exhibits varying phenotypes among genetically distinct groups (Silliman, 2019), which can influence their sensitivity to environmental stressors (Bible & Sanford, 2016; Heare, Blake, Davis, Vadopalas, & Roberts, 2017; Heare, White, Vadopalas, & Roberts, 2018; Maynard, Bible, Pespeni, Sanford, & Evans, 2018; Silliman, Bowyer, & Roberts, 2018). Indeed, the two groups to measure the response of *O. lurida* larvae to ocean acidification found contrasting results – no effect (Waldbusser *et al.*, 2016), and slower growth (Hettinger *et al.*, 2012, 2013) – possibly a result of local adaptation. The source population used for experimental studies may therefore be a critical factor influencing climate-related findings. Therefore, this study leveraged oysters from three phenotypically distinct Puget Sound populations, which were hatchery-reared in common conditions to adulthood (Heare *et al.* 2017, 2018).

Here, we investigate carryover effects of winter exposure to elevated temperature and high pCO₂ on reproduction and offspring viability across multiple *O. lurida* populations. This is the first study to assess the combined effects of elevated winter temperature and pCO₂ on reproduction, and the first to explore intergenerational carryover in an *Ostrea* spp. We exposed adult *O. lurida* to elevated temperature (+4°C), followed by elevated pCO₂ (+2204 µatm, -0.51 pH, Figure 2). Gonad development, reproductive timing, and fecundity were assessed for the adults, and offspring performance was assessed in the field. Elevated winter temperature was expected to impede gametogenic quiescence, presumably a critical annual event, subsequently

reducing larval production. This prediction was in part based on observations of low larval yields in an *O. lurida* restoration hatchery (*unpublished*) following the winter 2016 marine heat wave in the Northeast Pacific Ocean (Gentemann, Fewings, & García-Reyes, 2017). Similarly, we predicted that high pCO₂ exposure would result in negative impacts due to increased energy requirements for calcification and cellular maintenance. Finally, we predicted that negative impacts would be amplified upon exposure to both conditions. By assessing the effects of winter warming and acidification on reproduction and offspring viability in multiple Olympia oyster populations, we provide an ecologically relevant picture of how the species will respond to ocean change.

Methods

Adult oyster temperature and pCO₂ exposures

Four cohorts of adult *Ostrea lurida* were used in this study. Three of the cohorts were first-generation hatchery-produced (F1) oysters (32.1 ± 5.0 mm), all hatched in Puget Sound (Port Gamble Bay) in 2013 (Heare *et al.*, 2017). The broodstock used to produce these F1 oysters were wild, harvested from Fidalgo Bay in North Puget Sound (F), Dabob Bay in Hood Canal (D), and Oyster Bay in South Puget Sound (O-1) (O in Figure 1). These populations are considered genetically distinct subpopulations (Heare *et al.*, 2017; White, Vadopalas, Silliman, & Roberts, 2017). The fourth cohort (O-2, 21.9 ± 3.3 mm) was second-generation, hatchery-produced in 2015 from the aforementioned Oyster Bay F1 cohort, from a single larval release pulse and thus likely one family (Silliman *et al.* 2018). The O-2 cohort was included to examine whether reproductive and offspring traits were consistent across generations of a population, with the O-2 cohort being closely related to each other (siblings) and 2 years younger than the other cohorts.

145 Prior to the experiment, all oysters were maintained in pearl nets in Clam Bay (C) for a minimum
146 of 500 days.

147 **Temperature treatment**

148 Oysters were moved from Clam Bay (C) to the Kenneth K. Chew Center for Shellfish Research
149 and Restoration for the temperature and pCO₂ experiments. Oysters were held in one of two
150 temperature regimes (6.1±0.2°C and 10.2±0.5°C) for 60 days beginning December 6, 2016
151 (Figure 2). The temperatures correspond to historic local winter temperature (6°C) in Clam Bay,
152 and anomalously warm winter temperature (10°C) as experienced during 2014-2016 (Gentemann
153 *et al.*, 2017). For the temperature exposure, oysters from each cohort (100 for O-1 and F cohorts,
154 60 for D, and 300 for O-2) were divided into four bags, two bags per temperature, in two flow-
155 through experimental tanks (50L - 1.2-L/min). Temperature in the 6°C treatment was maintained
156 using a Teco Aquarium Chiller (TK-500), and unchilled water was used for the 10°C treatment.
157 Temperatures were recorded continuously with Onset HOBO Water Temperature Data Loggers
158 (U22-001).

159 **High pCO₂ treatment**

160 A differential pCO₂ exposure was carried out after the temperature treatment ended. Following a
161 10-day gradual temperature increase for the 6°C treatment to 10°C, oysters were further divided
162 and held at ambient pCO₂ (841±85 µatm, pH 7.82±0.02) or high pCO₂ (3045±488 µatm, pH 7.31
163 ± 0.02) for 52 days (February 16 to April 8, 2017, Figure 2). Animals were housed in six flow-
164 through tanks (50-L - 1.2-L/min), with three replicate tanks per pCO₂ treatment and oyster
165 cohort. High pCO₂ treated water was prepared using CO₂ injection. Filtered seawater (1µm) first

recirculated through a reservoir (1,610-L) with degassing column to equilibrate with the atmosphere, then flowed into treatment reservoirs (757-L) recirculating through venturi injectors. Durafet pH probes (Honeywell Model 51453503-505) and a Dual Input Analytical Analyzer (Honeywell Model 50003691-501) monitored pH in treatment reservoirs with readings every 180 seconds. Using solenoid valves, CO₂ gas was injected through lines at 15 psi in 0.4 second pulses if pH exceeded the 7.22 set point. Water pH was continuously monitored in experimental tanks using Durafet pH sensors, and temperature ($10.4 \pm 0.4^{\circ}\text{C}$) was measured using HOBO Pendant Temperature Data Loggers (UA-002-64). Twice weekly, water samples (1-L) were collected from experimental tanks, and temperature ($^{\circ}\text{C}$), salinity (PSU), and pH (mV, converted to pH_T) were measured immediately using Traceable Digital Thermometer (Model 15-077, Fisher), Bench/Portable Conductivity Meter (Model 23226-505, VWR), and a Combination pH Electrode (Model 11278-220, Mettler Toledo), respectively. Simultaneously, discrete water samples (120-mL) were collected in duplicate from experimental tanks and preserved with HgCl (50- μL) for later total alkalinity measurements using a T5 Excellence titrator (Mettler Toledo). Standard pH curves were generated on each sampling day prior to pH measurements using TRIS buffer prepared in-house at five temperatures (Supplementary Materials). Using the `seacarb` library in R, pCO₂, dissolved organic carbon (DIC), calcite saturation (Ω_{calcite}), and aragonite saturation ($\Omega_{\text{aragonite}}$) were calculated for days 5, 33, and 48 (Table 3, Supplementary Materials).

During both temperature and pCO₂ treatments, all oysters were fed from a shared algae header tank daily with Shellfish Diet 1800® (300-500-mL, Reed Mariculture) diluted in ambient pCO₂ seawater (200-L, Helm & Bourne, 2004), dosed continuously with Iwaki Metering Pumps. Twice weekly, experimental, reservoir, and algae tanks were drained and cleaned, and oysters were monitored for mortality and rotated within experimental system.

189 **Adult reproductive development**

190 A subset of oysters from each treatment were sampled for gonad stage and sex immediately
191 before and after pCO₂ treatments (Figure 2). Prior to pCO₂ exposure, 15 oysters were sampled
192 from O-1, O-2, and F cohorts, and 9 from D cohort. After pCO₂ exposure, 9, 6, and 15 oysters
193 were sampled from each treatment for O-1/F, D, and O-2 cohorts, respectively (distributed
194 equally among replicates tanks). Whole visceral mass was excised and preserved in histology
195 cassettes using the PAXgene Tissue FIX System, then processed for gonad analysis by
196 Diagnostic Pathology Medical Group, Inc. (Sacramento, CA).

197 Adult gonad samples were assigned sex and stage using designations adapted from (da
198 Silva, Fuentes, & Villalba, 2009) (Supplementary Materials). Sex was assigned as indeterminate
199 (I), male (M), hermaphroditic primarily-male (HPM), hermaphroditic (H), hermaphroditic
200 primarily-female (HPF), and female (F). Gonad sex was collapsed into simplified male and
201 female designations for statistical analyses (hermaphroditic-primarily male = male,
202 hermaphroditic-primarily female = female). For stage assignment, male and female gametes
203 were assigned stages separately due to the high frequency of hermaphroditism (50.8%).
204 Dominant gonad stage was then assigned based on the sex assignment. The da Silva gonad stages
205 were applied for early gametogenesis (stage 1), advanced (stage 2), and ripe (stage 3).
206 Departures from da Silva's stage 0, stage 4 (partially spawned), and stage 5 (fully
207 spawned/resorbing) were as follows: stage 0 in this study represented empty follicles, or no
208 presence of male or female gonad tissue; stage 4 represented both spawned and resorbing gonad;
209 this method did not include a separate stage 5, due to the very high frequency of residual
210 gametes, and no distinct partially spawned oysters (see Figure 3, and gonad images in
211 Supplementary Materials).

Treatment effects on gonad tissue were assessed for all cohorts combined in 4 gonad metrics: 1) gonad stage of dominant sex, 2) male gonad tissue when present, 3) female gonad tissue when present, and 4) gonad sex-collapsed (Chi-square test of independence). To assess the effects of elevated winter temperature alone, gonad metrics were compared between 6°C and 10°C treatments prior to pCO₂ treatment. To determine the effect of pCO₂ exposure, gonad metrics were compared between ambient and high pCO₂ after 52 days in pCO₂ treatments, including temperature interaction effects. To estimate whether gonad changed during pCO₂ treatment, metrics were compared before and after ambient and high pCO₂ treatments, including temperature interaction effects. P-values were estimated using Monte-Carlo simulations with 1,000 permutations, and corrected using the Benjamini & Hochberg method and $\alpha=0.05$ (Benjamini & Hochberg, 1995).

Larval production

Following pCO₂ exposure, adult oysters were spawned to assess larval production timing and magnitude in a hatchery setting. Beginning on April 11th (Figure 2), oysters were reproductively conditioned by raising temperatures gradually (~1°C/day) to $18.1 \pm 0.1^\circ\text{C}$ and fed live algae cocktail at $66,000 \pm 12,000$ cells/mL. Oysters were allowed to spawn volitionally in the hatchery for 90 days. Six spawning tanks were used for each temperature x pCO₂ treatment: 6°C-high pCO₂, 6°C-ambient pCO₂, 10°C-high pCO₂, and 10°C-ambient pCO₂. Within the six tanks per treatment, two spawning tanks contained the F cohort (14-17 oysters), two tanks the O-1 cohort (14-17 oysters), one tank the D cohort (9-16 oysters), and one tank the O-2 cohort (111-126 oysters). More O-2 oysters were used due to their small size. Olympia oysters are viviparous spermcasters and brood larvae to the veliger stage, so larvae were captured upon maternal

release. Spawning tank outflow was collected in 7.5-L buckets using 100 μm screens made from 15.25 cm polyvinyl chloride rings and 100 μm nylon mesh.

Larval collection was assessed for differences in spawn timing and fecundity. Larvae, first observed on May 11th (Figure 2), were collected from each spawning tank every one or two days for 60 days. Daily larval release was estimated by counting and averaging triplicate subsamples of larvae homogenized in seawater. The following summary statistics were compared between temperature x pCO_2 treatments: average daily larvae released, total larvae released, maximum larvae released in one day, date of first release, date of maximum release, and number of substantial release days (greater than 10,000 larvae). The total and daily release values were normalized by the number of broodstock * average broodstock height (cm), which can impact fecundity. Distributions were assessed using `qqp` in the `car` package for R (Fox & Weisberg, 2011), and log-transformed if necessary to meet normal distribution assumptions. Differences between treatments were assessed using linear regression and Three-Way ANOVA (cohort was included as a covariate) with backwards deletion to determine the most parsimonious models. Tukey Honest Significant Differences were obtained using `TukeyHSD` to assess pairwise comparisons (R Core Team, 2016). Dates of peak larval release were also estimated for each pCO_2 x temperature treatment by smoothing using locally weighted regression, with `geom_smooth` in the `ggplot` package (Wickham, 2017), with `span=0.3` and `degree=1`.

Offspring survival in a natural setting

To assess potential carryover effects of parental pCO_2 exposure, offspring from parents in 6°C-ambient pCO_2 and 6°C-high pCO_2 treatments were reared then deployed in the natural

environment. Larvae were collected between May 19 and June 22, 2017, separated by parental $p\text{CO}_2$ exposure and cohort, and reared in common conditions for approximately 1 year (Figure 2; for rearing methods see Supplementary Materials). On June 12, 2018 the juveniles were placed in four bays in Puget Sound —Fidalgo Bay, Port Gamble Bay, Skokomish River Delta, and Case Inlet — with two sites per bay, for a total of eight locations (Figure 1). Autonomous sensors collected continuous water quality data at each location for pH (Honeywell Durafet II Electrode, in custom-built housing), salinity (via conductivity, Dataflow Systems Ltd. Odyssey Conductivity and Temperature Logger), dissolved oxygen (Precision Measurement Engineering MiniDOT Logger), temperature (via dissolved oxygen probes), and chlorophyll (Turner Designs Cyclops-7F Submersible Sensor with PME Cyclops-7 Data Loggers). For F/D and O-1/O-2 cohorts, respectively, 30 and 10 oysters were placed at each location. Initial shell height and group weight were measured, then oysters were enclosed in mesh pouches and affixed inside shellfish bags to exclude predators. At the end of three months, survival, shell height and group weight were measured for live oysters.

Juvenile oyster survival was compared among bays and parental $p\text{CO}_2$ exposure with a binomial generalized linear mixed model (glmm) using `glmer` from the `lme4` package (vs. 1.1-19). Chi-square tests compared survival differences among factors using the `car` package `Anova` function (Fox & Weisberg, 2011). Mean shell growth was determined by subtracting pre-deployment mean height from post-deployment mean height (not including dead oysters), and compared among factors using ANOVA and F-statistics to test differences by bay and parental $p\text{CO}_2$. Similarly, mean mass change for each pouch was compared among factors.

All data analysis was performed in R version 3.3.1 using RStudio interface (R Core Team, 2016). Code for statistical analyses can be found in the associated Github repository (Spencer *et al.*, 2019).

Results

Adult reproductive development

After 60 days in temperature treatments ($6.1\pm0.2^{\circ}\text{C}$ and $10.2\pm0.5^{\circ}\text{C}$), gonad stage of the dominant sex differed significantly between temperatures (Table 2). The 10°C oysters had more instances of advanced gametogenesis (stage 2), and fewer resorbing/spawned (stage 4) (Figure 4, Supplementary Materials). This difference was influenced strongly by more advanced male gametes in 10°C oysters, but there were no differences in female gamete stages. No differences in sex were observed between temperature treatments (Figure 5).

After 52 days in pCO_2 treatments, gonad stage of the dominant sex differed significantly between ambient and high pCO_2 in the oysters previously held in 10°C (Table 2). More mature gametes (stage 3) were found in 10°C -ambient pCO_2 (49%) compared to 10°C -high pCO_2 (33%). This difference was strongly influenced by oysters that were predominantly male, as male gamete stage tended to differ between pCO_2 treatment, but female gamete stage did not (Table 2, Figure 4). In 6°C -treated oysters, there were no pCO_2 effects on gonad stage of the dominant sex, male gamete stage, or female gamete stage. No gonad stage or sex differences were detected among oysters from 10°C -high pCO_2 (combined stressors) and 6°C -ambient pCO_2 (no stressors). Gonad sex did not differ significantly among treatments, however oysters tended to contain fewer male-only and more female-only gonad tissues in the ripper, ambient pCO_2 -treated groups than male-only tissues (Figure 5).

301 Compared to oysters before pCO₂ exposure, those exposed to high pCO₂ did not differ in
302 gonad sex, stage of the dominant sex, or female gamete stage. Male gametes in the 6°C treated
303 oysters changed while in the high pCO₂ exposure, but not in 10°C treated oysters. Oysters held
304 in ambient pCO₂ had significantly more advanced gonad compared to before CO₂ exposure
305 regardless of temperature, again influenced strongly by changes in male gamete stage (Table 2).

306 No sampled oysters contained brooded embryos or larvae. Gonad data and patterns
307 within cohorts is reported in Supplementary Materials.

309 Larval production

310 Adults exposed to 10°C produced more larvae per day than 6°C in ambient pCO₂-exposed
311 oysters ($p=0.040$), but not in high pCO₂-exposed oysters ($p=0.66$) (Figure 7, pCO₂:temperature
312 interaction: $F(2,8)=5.1$, $p=0.037$). Total larvae released over the 90-day spawning period did not
313 differ by treatment (temperature:pCO₂ interaction $F(2,8)=4.0$, $p=0.063$). Temperature and pCO₂
314 as single factors did not affect total or average larval release.

315 The date of first larval release differed by temperature regardless of pCO₂ (Figure 6,
316 $F(1,8)=11.9$, $p=0.0087$), and pCO₂ had no effect on timing (not retained in model). Onset was on
317 average 5.2 days earlier in the 10°C treatment. Timing of peak larval release differed by
318 temperature treatment regardless of pCO₂ (Figure 7, $F(3,19)=6.7$, $p=0.018$), occurring on average
319 8.3 days earlier in 10°C oysters. The 10°C treated oysters produced more large pulses of larvae,
320 on average 2 additional days, than 6°C ($F(1,8)=7.25$, $p=0.027$).

321 In total, 18.5 million larvae were collected from 767 oysters. Total larvae produced by
322 each treatment was 3.1M, 4.8M, 5.9M, and 4.5M for 6°C-ambient pCO₂, 6°C-high pCO₂, 10°C-
323 ambient pCO₂, and 10°C-high pCO₂, respectively. Based on reports of approximately 215,000

larvae produced per adult *O. lurida* of shell height 35 mm (Hopkins, 1936), the number of oysters that spawned as female in this study was approximately 14.3, 22.5, 27.6, and 21.0 in the 6°C-ambient pCO₂, 6°C-high pCO₂, 10°C-ambient pCO₂, and 10°C-high pCO₂ treatments, respectively. This estimate is likely low across all treatments, due to the smaller D and O-2 cohorts (mean length in F, D, O-1 and O-2 was 35.7 mm, 29.8 mm, 35.7 mm, and 20.0 mm, respectively).

Larval production and timing data, including differences among cohorts, are included in the Supplementary Materials.

Offspring survival in a natural setting

Juvenile survival after three months in the field was on average 15% higher in cohorts from high pCO₂ exposed parents than from ambient pCO₂ parents (44±37%, and 29±27%, respectively, $\chi^2=10.6$, $p=0.0011$). The influence of parental pCO₂ on survival varied by bay (bay:parental pCO₂ interaction $\chi^2=15.3$, $p=1.6e-3$), and by cohort (cohort:parental pCO₂ interaction $\chi^2=23.5$, $p=3.2e-5$) (Table 3).

Survival in offspring from high pCO₂ parents was higher in the Fidalgo Bay and Port Gamble Bay locations ($\chi^2=17.7$, $p=2.6e-5$; $\chi^2=10.0$, $p=1.6e-3$, respectively), but this was not the case in Skokomish River Delta or Case Inlet. Survival in the F cohort was 38% higher in oyster from pCO₂ parents than those from ambient pCO₂ parents across all deployment bays ($\chi^2=28.1$, $p=4.6e-7$), and within the Fidalgo Bay location ($\chi^2=17.6$, $p\text{-adj}=0.0001$). Survival in the D and O-1 cohorts did not differ significantly between parental pCO₂ across all bays (D: $\chi^2=0.4$, $p=1$, O-1: $\chi^2=2.5$, $p=0.44$), or within individual bays. More O-2 juveniles with ambient

pCO₂ parents survived across all bays ($\chi^2=9.1$, $p=0.010$), and within the Skokomish River Delta ($\chi^2=8.9$, $p=0.011$).

Without considering parental pCO₂, more oysters survived in Port Gamble Bay (mean 49±36%) and Fidalgo Bay (39±36%) than in Case Inlet (mean 29±29%, $p=0.012$ & $p=0.037$, respectively) (bay factor, $\chi^2=18.5$, $p=3.4e-4$). Survival at Skokomish River Delta did not differ significantly from other locations (32±27%). No interaction between cohort and bay was detected ($\chi^2=9.8$, $p=0.37$) (Figure 8, Table 3).

Shell growth and mass per oyster were not affected by bay, cohort or parental pCO₂. However, due to varying mortality during deployment, comparisons between initial and final means were not likely accurate.

Discussion

Ocean acidification and ocean warming potentially threaten marine calcifiers and ectotherms, particularly those which are struggling to rebound after population crashes, such as the Olympia oyster. An organism's genotype, complete environmental history, and the timing and magnitude of environmental perturbations may all determine its fitness in future ocean conditions. To begin teasing apart these complex factors, this study leveraged four adult Olympia oyster cohorts with distinct genetic structure but known, shared histories. Elevated winter temperature resulted in increased gonad development, which corresponded with earlier and more frequent larval release (on average 5.2 days earlier, 2 additional days). High pCO₂ exposure negatively influenced gonad maturation state, but did not affect subsequent fecundity. Offspring from parents exposed to elevated pCO₂ had higher overall survival upon deployment. Differences in juvenile survival bays and cohorts indicate that carryover effects are dependent upon the

environment and genotype, and reinforce the importance of using multiple sources of test organisms in stress-response studies.

Reproduction

We expected elevated winter temperature to reduce fecundity, based on predictions that changes to reproductive quiescence and metabolism would be deleterious to spring reproduction. Counter to this prediction, warm winter temperature positively affected larval production, possibly due to uninterrupted spermatogenesis. Oysters in elevated temperature contained more developed male gametes after treatment, and subsequently began releasing larvae earlier and produced more larvae per day, compared to cold-treated oysters. We find no evidence that cold winters are critical for spring reproduction, but rather elevated winter temperature may elongate the *O. lurida* spawning season. In comparison, a 29-year dataset of *M. balthica* reproduction showed that as winter temperature increased, spring spawning began earlier and fecundity declined (Philippart *et al.*, 2003). This study was conducted in a hatchery setting, with ample phytoplankton, and did result in a temperature shift during spawning, which should be considered. In the wild numerous additional abiotic and biotic factors will contribute to *O. lurida* fitness, and warmer winters may result in earlier and longer reproductive seasons only if nutritional requirements are met. Whether larvae released earlier in the spring can survive to recruitment will greatly depend on many things including food availability and predation.

We predicted that high pCO₂ exposure would redirect energy away from storage to maintenance processes, resulting in delayed gametogenesis and poor fecundity in the spring. After exposure to 3045 μ atm pCO₂ (pH 7.31), fewer oysters contained ripe or advanced male gonad tissue than in ambient pCO₂, signaling reduced spermatogenic activity. Female gonad, sex ratios, and subsequent fecundity were not affected by sole exposure to high pCO₂. Similar

impacts on gametogenesis during exposure were observed in the Sydney rock (*S. glomerata*) and Eastern (*C. virginica*) oysters, but with varying pCO₂ thresholds. Parker *et al.* (2018) found *S. glomerata* gametogenesis to slow in 856 µatm (pH 7.91), and Boulais *et al.* (2017) found normal rates at 2260 µatm (pH 7.5), delay at 5584 µatm (pH 7.1), and full inhibition at 18480 µatm (pH 6.9) in *C. virginica*. Together, these studies indicate that high pCO₂ slows the rate of gametogenesis, but the level at which pCO₂ affects gametogenesis appears species-specific, and likely reflective of variable physiological mechanisms and reproductive strategies.

The combined effects of sequential elevated temperature and pCO₂ treatments did not act synergistically to delay gonad development, but instead resulted in oysters with gonad stage and fecundity no different from the untreated oysters. Similarly, combined simultaneous temperature and high pCO₂ exposures did not affect *S. glomerata* fecundity (Parker *et al.*, 2018). We did detect a pCO₂ dependent effect of temperature on the average number of larvae released per day. Oysters that had previously been exposed to 10°C produced more larvae than 6°C, but only after ambient pCO₂ exposure, which may reflect a general reproductive arrest that occurs when exposed to high pCO₂. These preliminary dual-stressor studies indicate that high pCO₂ slows gametogenesis, elevated temperature accelerates it, and these two environmental drivers act antagonistically on gonad development if occurring in the same reproductive season.

In contrast to prior studies, temperature and pCO₂ did not impact *O. lurida* sex ratios, whereas in high pCO₂ *C. virginica* skewed male (Boulais *et al.*, 2017), and *S. glomerata* skewed female (Parker *et al.*, 2018). This observation may be explained by very low incidence of total reproductive inactivity in our *O. lurida* cohorts — only four out of the 108 oysters that were sampled prior to pCO₂ treatment contained empty follicles — and thus sex ratios may be different if pCO₂ exposure occurs earlier in life during initial sex differentiation. Furthermore,

high pCO₂ exposure only occurred in winter, prior to spawning. If high pCO₂ persists during oocyte maturation and spawning, *O. lurida* fecundity may be reduced similar to *C. virginica* and *S. glomerata*. Future research should examine *O. lurida* spawning and fertilization in first-year juveniles across a range of pCO₂ to determine conditions in which gametogenesis and sex determination are affected.

Offspring

Abiotic parental stressors can be beneficial, neutral, or detrimental to offspring viability (Donelson *et al.*, 2018). We explored carryover effects of adult exposure to winter pCO₂ on offspring by testing survival in the field. Offspring with high pCO₂ parental histories performed better in two of four locations, Fidalgo Bay and Port Gamble Bay. Carryover effects of parental high pCO₂ exposure may therefore be neutral, or beneficial, to offspring depending on the environmental conditions. Port Gamble Bay and Fidalgo Bay are more influenced by oceanic waters, which could explain cooler observed temperatures. These locations are also typically less stratified than the Skokomish River Delta and Case Inlet. In Port Gamble Bay, where pCO₂ parental history most significantly correlated with offspring survival across cohorts, mean pH was considerably lower than the other deployment locations (-0.17 pH units), and mean salinity was higher (+3.8 PSU). Given the experimental design we are able to clearly demonstrate that manifestation of carry-over effects in Olympia oysters is dependent on environmental conditions. Specifically, there is a greater likelihood of beneficial carryover effects when parents are exposed to stressful conditions. Overall, carryover effects of parental pCO₂ treatment were positive, however negative effects were observed in the O-2 cohort. This discrepancy could relate to unique O-2 juvenile characteristics, as they were bred from siblings, were 3rd-

generation hatchery produced, and varied in size. The complex interactions among parental exposure, bay, and cohort indicate that offspring viability is influenced by ancestral environment history, environmental conditions, and genotype.

Our results contrast a similar study that exposed *C. gigas* oysters to high pCO₂ during the winter, three months prior to reproductive conditioning. They found that exposed females produced fewer hatched larvae 18 hours post-fertilization, with no discernable paternal effect (Venkataraman *et al.*, 2019). Hatch rate was not directly measured in this study due to the *O. lurida* brooding behavior; however, no difference in daily and total larvae released suggest that hatch rate was unaffected. The different responses may reflect variability among species and spawning method. Venkataraman *et al.* (2019) artificially collected gametes by stripping gonad, whereas *O. lurida* late-stage veliger larvae were collected upon release from the brood chamber. Volitionally-spawned gametes could be higher quality than those strip-spawned. Larval brooding may also be a mechanism by which sensitive larvae are acclimatized to stressors, as the *O. lurida* brood chamber pH and dissolved oxygen can be significantly lower than the environment (Gray *et al.*, *in press*).

Beneficial parental carryover may also be linked to the male-specific gonad effects, and the conditions in which the adult oysters were held. During pCO₂ treatments, there was little change in female development and no difference in female gamete stage between pCO₂ treatments. Negative intergenerational carryover effects are commonly linked to variation in oocyte quality, which can be affected by the maternal environment (Utting & Millican, 1997). In the Chilean flat oyster (*Ostrea chilensis*), egg size and lipid content positively correlate with juvenile growth and survival (Wilson, Chaparro, & Thompson, 1996). If high pCO₂ were to coincide with rapid proliferation of oocytes and final maturation, *O. lurida* egg quality and larval

viability could be compromised. In contrast, male gonad stage advanced significantly during pCO₂ exposure. Intergenerational and transgenerational carryover effects are increasingly linked to the paternal environment in other taxa, such as inheritance of epigenetic changes to the male germ line (Rodgers, Morgan, Bronson, Revello, & Bale, 2013; Skinner, 2007; Soubry, Hoyo, Jirtle, & Murphy, 2014). Positive carryover effects of environmental stressors observed in this and other marine invertebrate taxa may be due to paternal epigenetic effects, but this link has not yet been observed.

This study clearly demonstrates exposure to elevated winter temperature and altered carbonate chemistry impacts reproduction and offspring viability in the Olympia oyster. Furthermore, we report the first observations of intergenerational plasticity in an *Ostrea* species, that is dependent on offspring environmental conditions and population. This characteristic could have a substantial impact on species resilience. With these considerations, future biological response studies need to be aware of three possible factors influencing results: 1) source population; 2) the source population's environmental history (within its lifetime); and 3) the source population's ancestral environmental history (inter and transgenerational carryover effects). Controlling for, or at minimum recognizing and recording these factors, will provide important context for those predicting ecosystem response to environmental change.

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Table 1: Environmental data from locations where offspring were deployed for 3 months. Mean \pm SD of continuously monitored environmental data are shown for periods of tidal submergence only (tidal height >0.3m), collected at two deployment locations within each bay.

	Fidalgo Bay	Port Gamble Bay	Skokomish River Delta	Case Inlet
Temperature (°C)	15.4 \pm 1.5	15.0 \pm 1.0	16.2 \pm 2.7	16.8 \pm 1.7
DO (mg/L)	10.6 \pm 2.4	10.5 \pm 1.9	10.2 \pm 3.9	11.2 \pm 2.8
Salinity (PSU)	28.5 \pm 3.9	31.9 \pm 2.0	29.6 \pm 1.3	24.6 \pm 1.7
pH	8.07 \pm 0.15	7.86 \pm 0.17	8.01 \pm 0.20	8.01 \pm 0.16
chlorophyll	227 \pm 409	225 \pm 145	572 \pm 1536	331 \pm 613

Table 2: Pearson's chi-square test results comparing gonad sex and stage among treatments. Gonad was sampled after temperature treatment but before pCO₂ (6°C Pre and 10°C Pre, n=54), and after pCO₂ treatment (Amb=841±85 µatm, n=39; High= 3045±488 µatm, n=39). Chi-square results are shown for gonad sex, stage of the dominant sex, male gametes when present, and female gametes when present. Bottom triangle = Pearson's chi-square value, top triangle = p-adjusted. Values in bold indicate significant differences between comparison; x=not tested; % of mature = % of sampled oysters that contained stage 3 male or female gametes, per treatment.

	6°C Pre	10°C Pre	6°C Amb	6°C High	10°C Amb	10°C High	6°C Pre	10°C Pre	6°C Amb	6°C High	10°C Amb	10°C High
	<i>Sex</i>						<i>Stage of the dominant sex</i>					
6°C Pre	-	0.26	0.93	0.34	x	x	-	0.017	0.013	0.48	x	x
10°C Pre	5.9	-	x	x	0.18	0.46	15.8	-	x	x	0.038	0.44
6°C Amb	0.8	x	-	0.29	x	0.29	16.5	x	-	0.090	x	0.78
6°C High	4.6	x	5.4	-	x	x	4.6	x	9.7	-	x	x
10°C Amb	x	6.8	x	x	-	0.94	x	12.7	x	x	-	0.038
10°C High	x	3.8	5.3	x	0.6	-	x	5.2	2.8	x	12.5	-

	<i>Male gametes</i>						<i>Female gametes</i>					
6°C Pre	-	1.6e-3	1.6e-3	0.013	x	x	-	0.78	0.18	0.47	x	x
10°C Pre	31.1	-	x	x	0.038	0.95	2.1	-	x	x	0.26	0.17
6°C Amb	24.2	x	-	0.071	x	0.78	6.3	x	-	0.36	x	0.9
6°C High	15.2	x	9.0	-	x	x	3.6	x	4.4	-	x	x
10°C Amb	x	11.2	x	x	-	0.084	x	4.2	x	x	-	1
10°C High	x	0.6	1.7	x	9.5	-	x	5.5	0.8	x	0.15	-
% mature	30%	19%	28%	15%	33%	21%	2%	6%	15%	8%	18%	21%

Table 3: Offspring survival in the field. 1-year old juveniles were deployed for 3 months in four bays in Puget Sound, Washington, in 2 sites per bay. Percent survival \pm SD is shown by cohort \times bay \times parental pCO₂ treatment (Amb=841 \pm 85 μ atm, High= 3045 \pm 488 μ atm). Only offspring from 6°C-treated adults were deployed. Values in bold indicate significant survival difference by parental pCO₂ treatment. Mean shell height \pm SD before and after deployment is shown.

Cohort \rightarrow	Fidalgo Bay (F)		Dabob Bay (D)		Oyster Bay F1 (O-1)		Oyster Bay F2 (O-2)		All cohorts	
pCO ₂ \rightarrow Bay \downarrow	Amb	High	Amb	High	Amb	High	Amb	High	Amb	High
All Bays	27 $\pm 22\%$	62 $\pm 29\%$	30 $\pm 22\%$	34 $\pm 28\%$	38 $\pm 37\%$	58 $\pm 41\%$	20 $\pm 16\%$	4 $\pm 13\%$	29 $\pm 27\%$	44 $\pm 37\%$
Fidalgo	20 $\pm 32\%$	85 $\pm 10\%$	22 $\pm 12\%$	38 $\pm 25\%$	40 $\pm 46\%$	62 $\pm 43\%$	11 $\pm 15\%$	13 $\pm 23\%$	25 $\pm 30\%$	51 $\pm 37\%$
Port Gamble	33 $\pm 27\%$	74 $\pm 17\%$	35 \pm 35 %	63 $\pm 21\%$	40 $\pm 47\%$	93 $\pm 12\%$	21 $\pm 0\%$	0%	34 $\pm 33\%$	64 $\pm 34\%$
Skokomish	32 $\pm 17\%$	51 $\pm 23\%$	45 $\pm 11\%$	18 $\pm 13\%$	20 $\pm 28\%$	35 $\pm 41\%$	33 $\pm 24\%$	0%	32 $\pm 21\%$	31 $\pm 33\%$
Case Inlet	20 $\pm 19\%$	40 $\pm 30\%$	18 $\pm 15\%$	15 $\pm 26\%$	50 $\pm 26\%$	50 $\pm 48\%$	14 $\pm 20\%$	0%	27 $\pm 23\%$	30 $\pm 35\%$
initial shell height (mm)	9.1 ± 2.3	8.4 ± 2.9	7.0 ± 1.4	6.3 ± 1.4	11.2 ± 3.0	11.0 ± 3.4	11.0 ± 3.3	7.5 ± 2.9	9.6 ± 3.1	8.6 ± 3.2
final shell height (mm)	20.6 ± 5.9	20.5 ± 5.2	17.9 ± 3.5	16.1 ± 4.1	21.8 ± 5.9	21.8 ± 4.7	23.7 ± 3.1	9.3 ± 0.2	20.2 ± 5.2	19.4 ± 5.3

Figure 1: Locations where *O. lurida* populations' progenitors were collected (F, D, O), where oysters were housed prior to and during the experiment (C), and where offspring were deployed (F, P, S, I): Fidalgo Bay (F), Port Gamble Bay (P), Dabob Bay (D), Clam Bay (C), Skokomish River Delta (S), Case Inlet (I), Oyster Bay (O).

Figure 2: Experimental timeline. Four cohorts of adult *O. lurida* (F, D, O-1, O-2) were sequentially exposed to two winter temperatures ($6.1 \pm 0.2^\circ\text{C}$, $10.2 \pm 0.5^\circ\text{C}$) then two pCO_2 levels ($841 \pm 85 \mu\text{atm}$, $3045 \pm 488 \mu\text{atm}$). They were returned to ambient pCO_2 conditions to volitionally spawn. Larvae were collected and reared by cohort x temperature x pCO_2 . Juveniles (~ 1 year) from 6°C -Ambient pCO_2 and 6°C -Low pCO_2 adults were deployed in 4 bays in Puget Sound.

Figure 3: Examples of *Ostrea lurida* gonad stage designations. Stage 0 (no activity/sex differentiation); Stage 1 (early gametogenesis); Stage 2 (advanced gametogenesis); Stage 3 (late gametogenesis / ripe); Stage 4 (spawned and/or resorbing).

Figure 4: Gonad developmental stages for male and female gametes, after 60-days in temperature treatments but before pCO_2 treatments ("Pre", $n=54$) and after 52 days in high pCO_2 ($3045 \pm 488 \mu\text{atm}$, $n=39$) and ambient pCO_2 ($841 \pm 85 \mu\text{atm}$, $n=39$). All oysters were assigned both male & female stages; if no oocytes were present, for example, that oyster was designated as female stage 0.

Figure 5: Gonad sex, after 60-days in temperature treatments but before pCO_2 treatments ("Pre", $n=54$) and after 52 days in high pCO_2 ($3045 \pm 488 \mu\text{atm}$, $n=39$) and ambient pCO_2 ($841 \pm 85 \mu\text{atm}$, $n=39$).

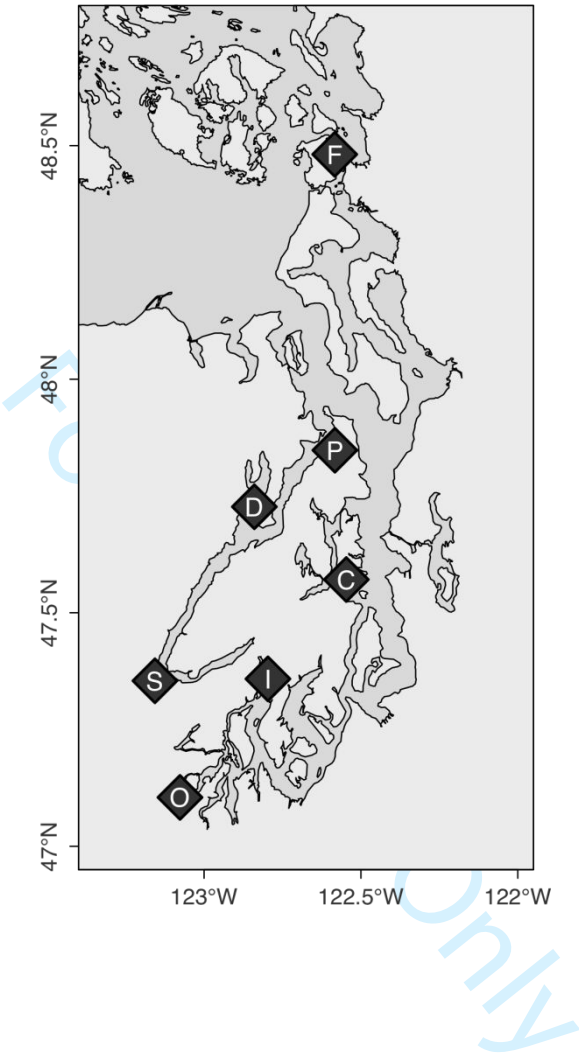
Figure 6: Cumulative larvae released over 90 days of continuous volitional spawning under hatchery conditions. Each of the four panels represent a cohort, and lines are color coded by winter temperature and pCO₂ treatments, where ambient pCO₂ = 841 µatm (7.8 pH), and high pCO₂ = 3045 µatm (7.31). Reproductive conditioning and spawning occurred at 18°C, in ambient pCO₂, and with live algae at a density of 66,000 ± 12,000 cells/mL.

Figure 7: Left: mean larvae released per day, normalized by number of oysters * average oyster height (cm). Daily production was higher in 10°C than 6°C, but only in oysters exposed to ambient pCO₂. Right: number of spawning days until larval release peaked; peak release occurred earlier in 10°C treated oysters. Letters (a, ab, b) indicate differences among treatments. Boxes contain values lying within the interquartile range (IQR), with medians indicated by lines in the middle of boxes. Whiskers extend to the largest value no greater than 1.5*IQR.

Figure 8: Percent survival of juvenile offspring in the field. The four panels each represent survival in one bay (Fidalgo Bay, Port Gamble Bay, Skokomish River Delta, Case Inlet). Within each panel, boxplots are separated by parental pCO₂ exposure (Ambient=841 µatm, High=3045 µatm). Points indicate % survival in each deployment pouch, and symbols indicate cohort (Fidalgo Bay, Dabob Bay, Oyster Bay Cohort 1, and Oyster Bay Cohort 2). Letters (a, b) indicate survival differences among parental pCO₂ exposure within each bay. Boxes contain values lying within the interquartile range (IQR), with median survival indicated by lines in the middle of boxes. Whiskers extend to the largest value no greater than 1.5*IQR.

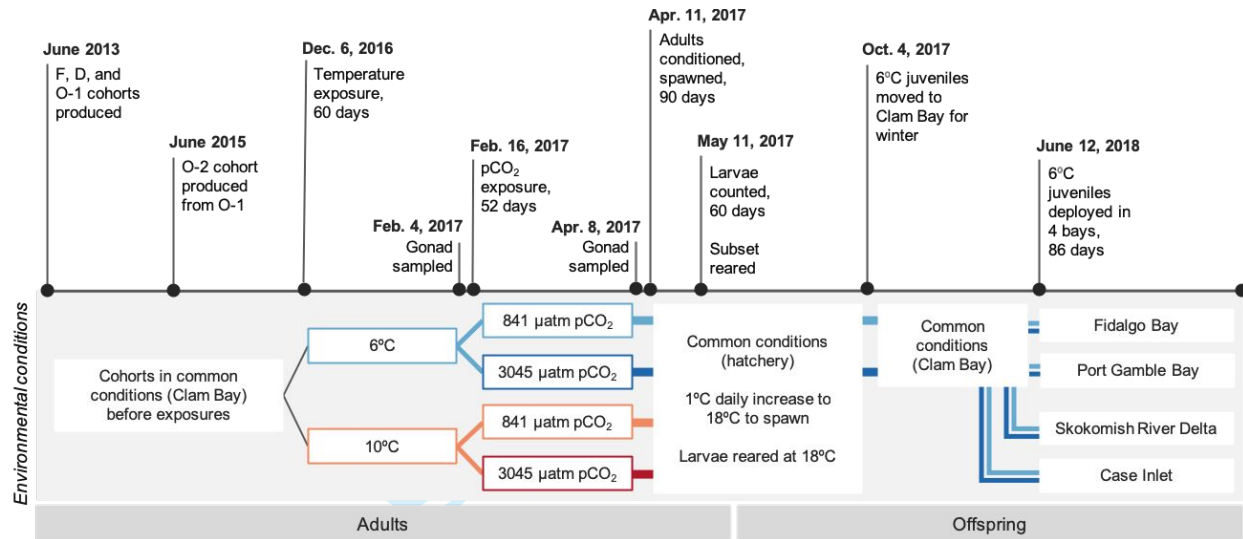
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Figure 1



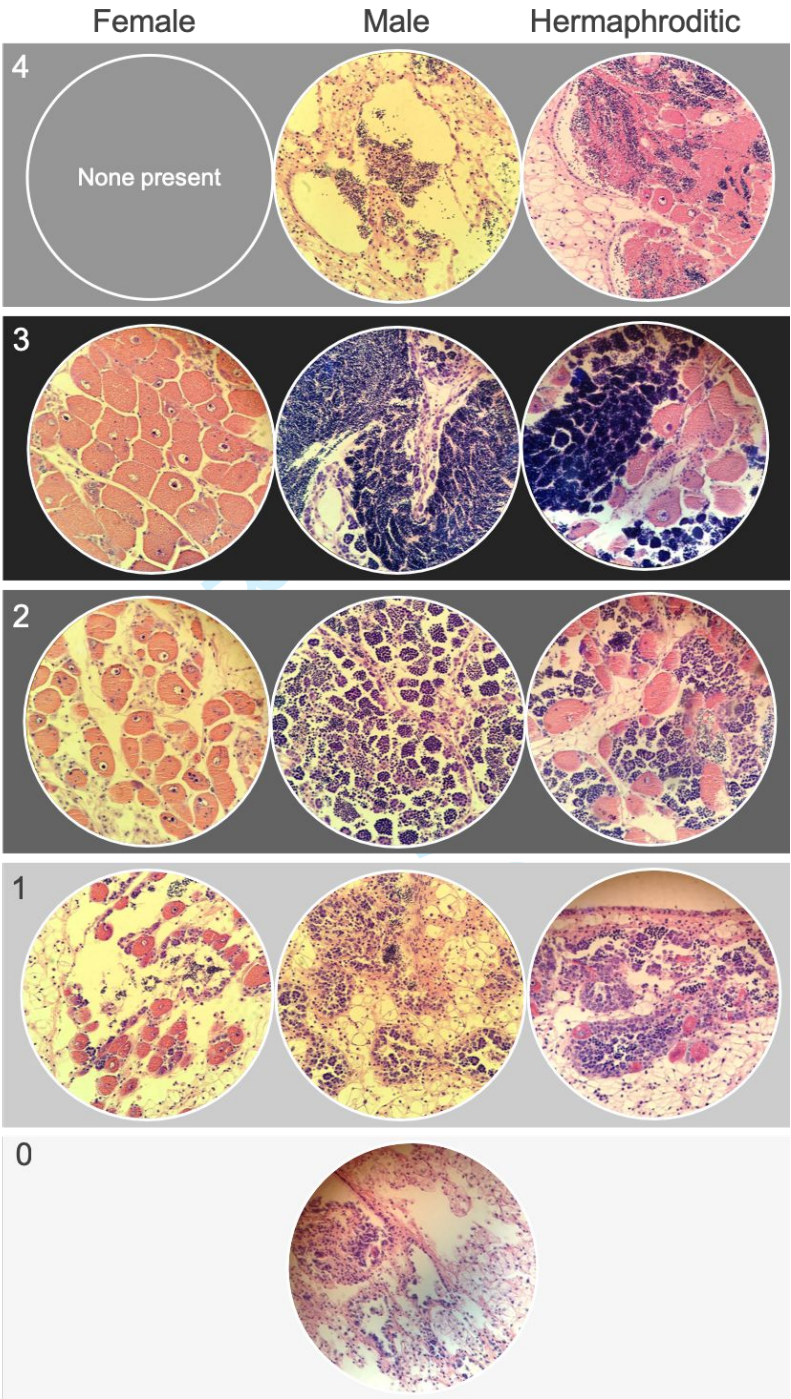
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Figure 2

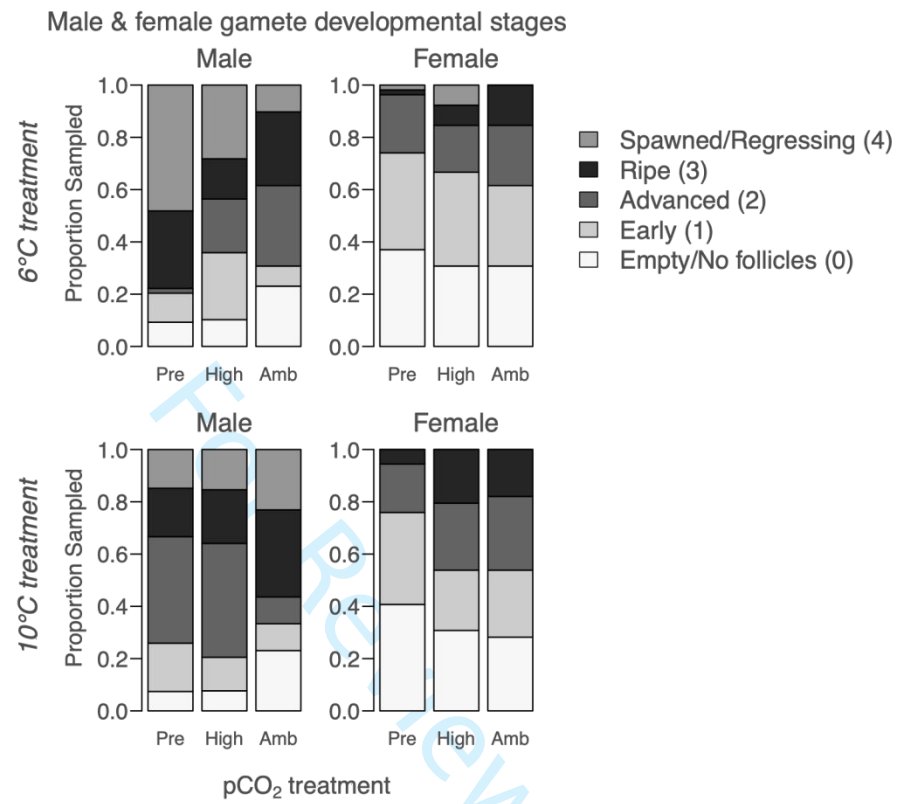


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Figure 3

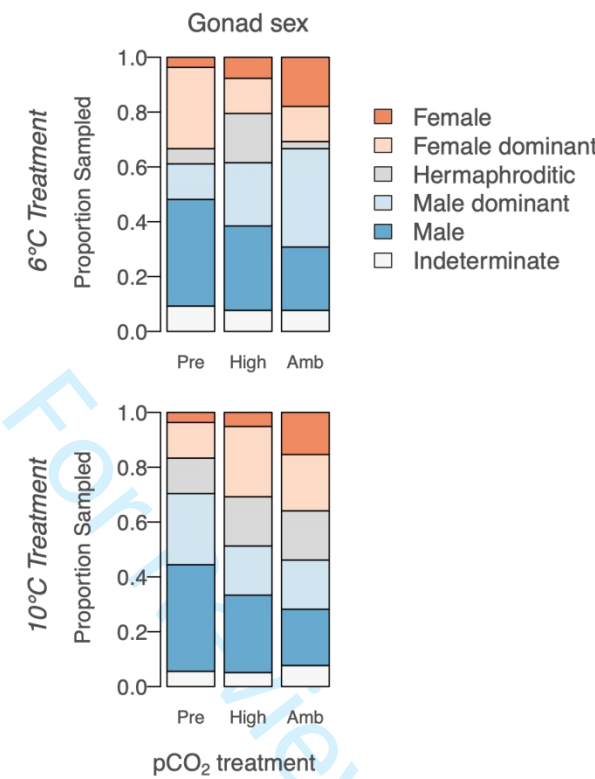


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Figure 4

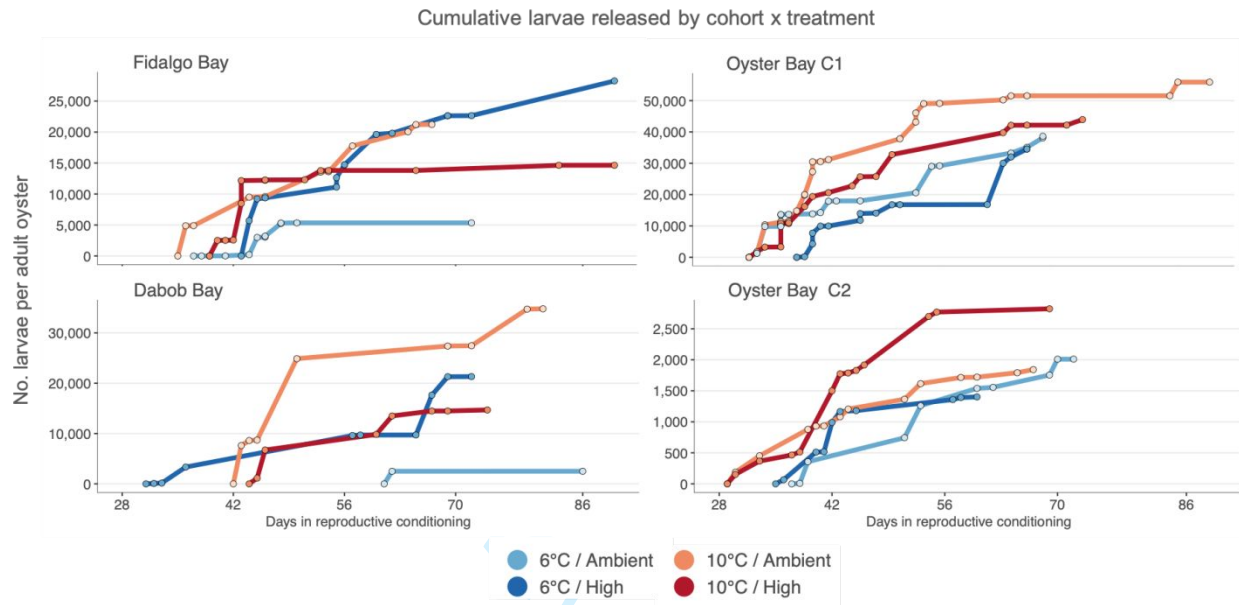
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Figure 5



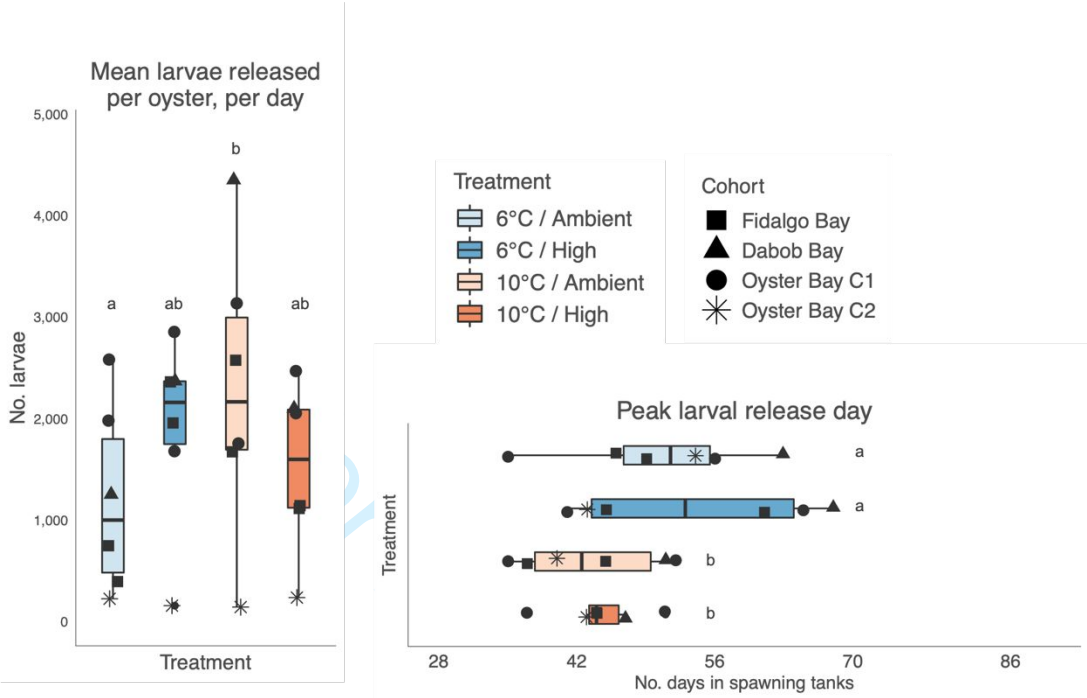
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Figure 6

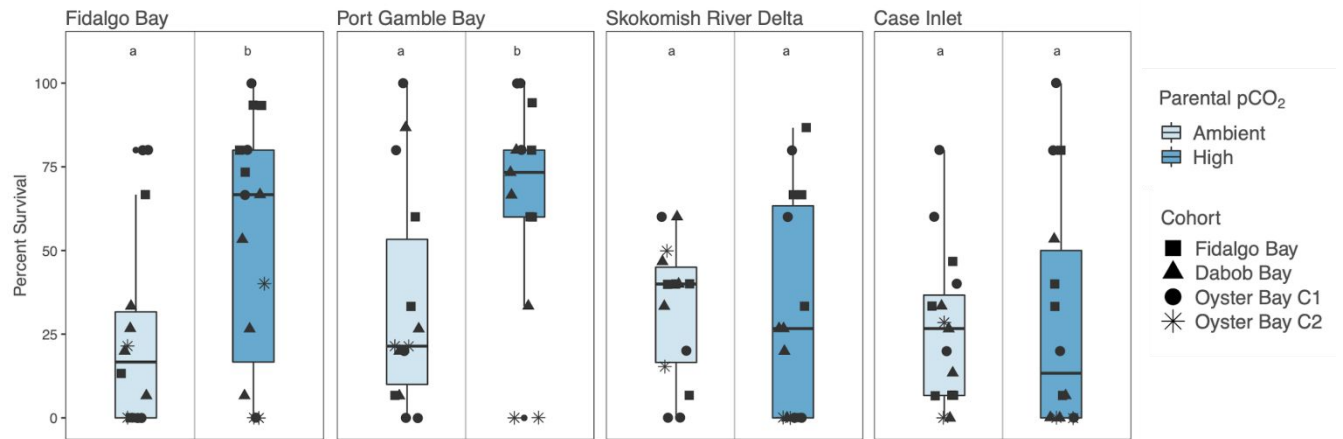


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Figure 7



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Figure 8

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Supplementary Materials

Supplementary Table 1: Gonad stage designations, adapted from da Silva 2009

Stage	Designation	Description
0	Inactive	Empty follicles, no presence of male or female gonad tissue.
1	Early gametogenesis	Gametes were mostly attached to the follicle wall. In the male developing line, spermatogonia and spermatocytes, very few spermatids; in the female developing line, mostly attached, developing ovogonia and early ovocytes.
2	Advanced gametogenesis	In the male developing line few spermatogonia, spermatocytes and spermatid balls were dominant, and few spermatozoa balls appeared in the follicle lumen; in the female developing line, few ovogonia present, ovocytes in vitellogenesis but attached were dominant, and ovocytes in post-vitellogenesis and located free in the follicle lumen less abundant.
3	Ripe	In both male and female developing lines, follicles contained mature gametes and sometimes a thin layer of primary germ cells. Abundant spermatozoa balls and mature ovocytes filled the follicle lumen, in male line and female line, respectively.
4	Spawned (full or partial), and/or resorbing	Gametes had been released, and follicles are dilated but lumen was empty, or contained residual mature gametes; residual oocytes of various sizes were sparsely distributed; residual spermatids were dissociated within follicle lumen. In some cases phagocytes were observed within follicles to re-absorb residual gametes. In many cases residual gametes of one sex remained, while developing gametes of the other sex were abundant.

Supplementary Table 2: Gonad sex designations, from da Silva 2009

Sex (uncollapsed, from da Silva)	Sex, collapsed for statistical analyses	Designation	Description
F	F	Female	Follicles contain only female gonad material (any stage)
HPF	F	Hermaphroditic, predominantly female	Follicles contain predominantly female but also some male gonad material
H	H	Hermaphroditic	Follicles contain approximately half male and half female gonad material
HPM	M	Hermaphroditic, predominantly male	Follicles contain predominantly male but also some female gonad material
M	M	Male	Follicles contain only male gonad material (any stage)
I	I	Indeterminate	Follicles are empty, collapsed, or only undifferentiated gonidia are visible

Supplementary Table 3: Carbonate chemistry parameters for three time points during the pCO₂ treatments, which are averages (\pm SE) from three replicate tanks per treatment. All parameters except for total alkalinity differed significantly between control/ambient (Amb.) and experimental/high (High.) tanks (One-way ANOVA). More details are available in Venkataraman *et al.*, 2019.

Day	pH*** F(1,16) = 5838, p = 6.12e-22		Total Alkalinity (μ mol/kg) F(1,16) = 1.38, p = 0.257		pCO ₂ (μ atm)*** F(1,16) = 235, p = 5.44e-11		DIC (μ mol/kg)* F(1,16) = 7.12, p = 0.0168		Ω_{calcite} *** F(1,16) = 529, p = 1.10e-13		$\Omega_{\text{aragonite}}$ *** F(1,16) = 527, p = 1.14e-13	
	Amb.	High	Amb.	High	Amb.	High	Amb.	High.	Amb.	High	Amb.	High
5	7.82 \pm 0.004	7.33 \pm 0.002	2307.41 \pm 25.45	2332.36 \pm 31.05	747.51 \pm 13.94	2481.23 \pm 29.83	2233.41 \pm 25.29	2408.51 \pm 31.76	1.86 \pm 0.02	0.62 \pm 0.01	1.16 \pm 0.012	0.58 \pm 0.007
33	7.81 \pm 0.005	7.31 \pm 0.004	2747.00 \pm 21.13	2917.60 \pm 18.36	912.22 \pm 12.69	3309.52 \pm 7.22	2664.57 \pm 19.99	3020.99 \pm 17.99	2.23 \pm 0.03	0.77 \pm 0.02	1.40 \pm 0.020	0.48 \pm 0.014
48	7.82 \pm 0.015	7.29 \pm 0.004	2611.40 \pm 31.01	2808.39 \pm 12.24	863.47 \pm 42.42	3343.89 \pm 49.49	2533.28 \pm 35.45	2920.52 \pm 15.11	2.13 \pm 0.06	0.68 \pm 0.01	1.32 \pm 0.035	0.42 \pm 0.004

Recipe for Tris buffer (0.08 M, 28.0 PSU):

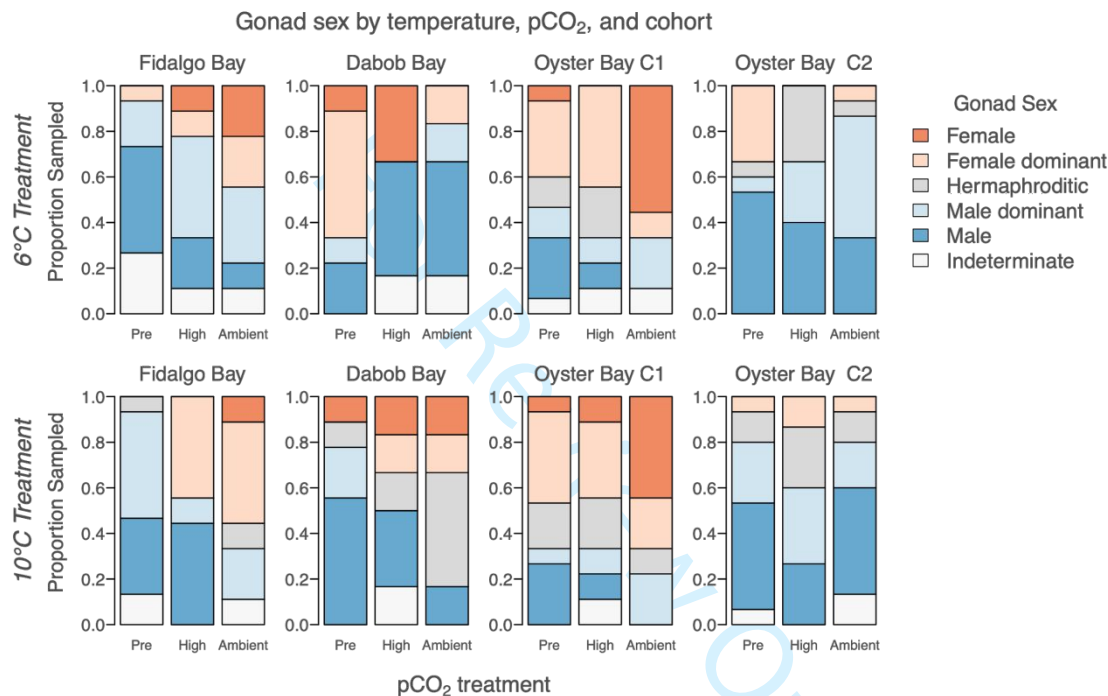
- 0.3603 mol of NaCl (J.T. Baker)
- 0.0106 mol of KCl (Fisher Scientific)
- 0.0293 mol MgSO₄·(H₂O)₇ (Fisher Scientific)
- 0.0107 mol of CaCl₂·2(H₂O) (MP Biomedicals)
- 0.0401 HCl (J.T. Baker)
- 0.0799 mol of Tris base (Fisher Scientific)
- Deionized water was added for a final volume of 1L

Supplementary Table 4: Frequency of dominant gonad stages by cohort and pCO₂ exposure, separated by winter temperature treatment (6°C, 10°C). Results of Chi-Square between pCO₂ treatments are shown as p-values for dominant stage (Dom-stage), dominant sex (Dom-sex), Male stages (Male), and female stages (female). Across all cohorts in both 6°C and 10°C treatments, dominant stage and male stage differed by pCO₂ treatments. Stage differences were detected between pre-treatment (pre-pCO₂) and after treatment for ambient and high pCO₂ separately, and are indicated by superscripts A and B: A= pre-treatment and ambient pCO₂ differed; B=pre-treatment and high pCO₂ differed (p<0.05). Stage and sex comparisons in bold indicate that they were significantly different between temperature treatments, prior to pCO₂ treatments (“Pre” columns).

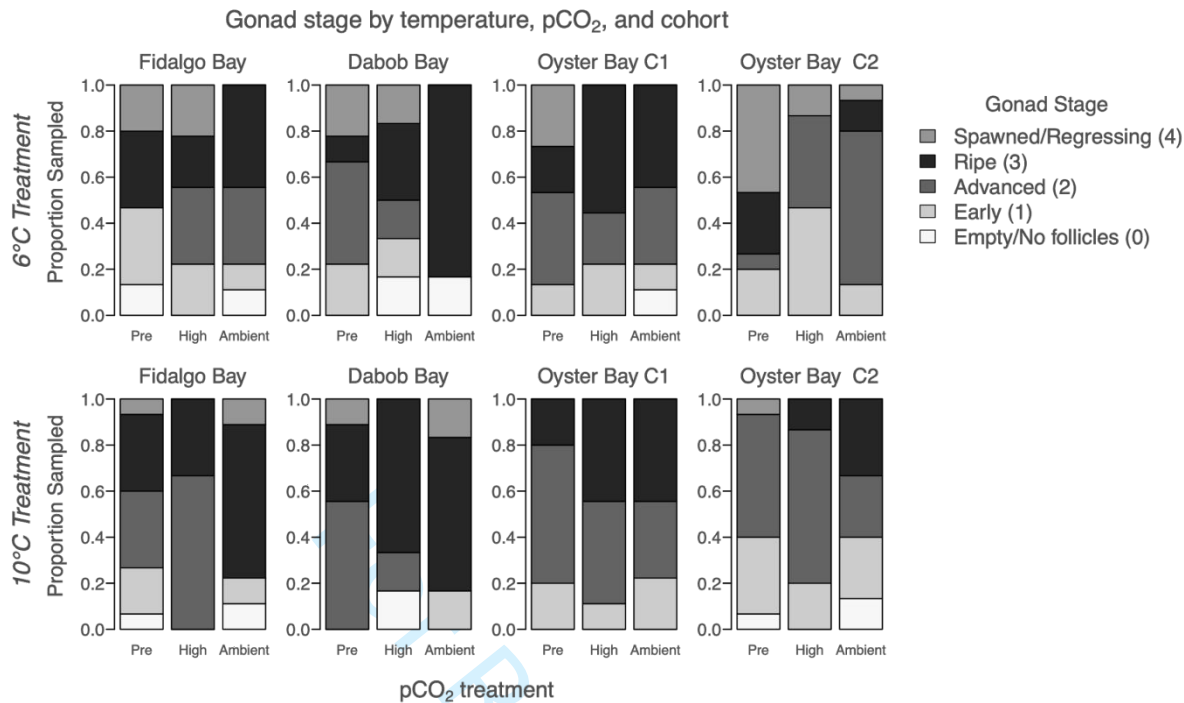
<i>Dominant gonad stage for 6°C treatment, by cohort and pCO₂ exposure</i>															
	<i>Fidalgo Bay</i>			<i>Dabob Bay</i>			<i>Oyster Bay C1</i>			<i>Oyster Bay C2</i>			<i>All cohorts</i> Dom-stage^A : p=0.036 <i>Dom-sex^A</i> : p=0.202 Male^{A,B} : p=0.030 <i>Female</i> : p=0.219		
<i>Gonad Stage</i>	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂
Spawned / Resorbing (4)	3	2	0	2	1	0	4	0	0	7	2	1	16	5	1
Ripe (3)	5	2	4	1	2	5	3	5	4	4	0	2	13	9	15
Advanced (2)	0	3	3	4	1	0	6	2	3	1	6	10	11	12	16
Early (1)	5	2	1	2	1	0	2	2	1	3	7	2	12	12	4
Empty Follicles (0)	2	0	1	0	1	1	0	0	1	0	0	0	2	1	3
<i>Total Sampled</i>	15	9	9	9	6	6	15	9	9	15	15	15	54	39	39
<i>Dominant gonad stage for 10°C treatment, by cohort and pCO₂ exposure</i>															
	<i>Fidalgo Bay</i>			<i>Dabob Bay</i>			<i>Oyster Bay C1</i>			<i>Oyster Bay C2</i>			<i>All cohorts</i> Dom-stage^A : p=0.008 <i>Dom-sex^A</i> : p=0.73 Male^A : p=0.020 <i>Female</i> : p=1		
<i>Gonad Stage</i>	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂	Pre pCO ₂	High pCO ₂	Amb pCO ₂
Spawned / Resorbing (4)	1	0	1	1	0	1	0	0	0	1	0	0	3	0	2
Ripe (3)	5	3	6	3	4	4	3	4	4	0	2	5	11	13	19
Advanced (2)	5	6	0	5	1	0	9	4	3	8	10	4	27	21	7
Early (1)	3	0	1	0	0	1	3	1	2	5	3	4	11	4	8
Empty Follicles (0)	1	0	1	0	1	0	0	0	0	1	0	2	2	1	3
<i>Total Sampled</i>	15	9	9	9	6	6	15	9	9	15	15	15	54	39	39

Gonad sex and stage details and cohort traits

Of all sampled oysters, 53.4% were hermaphrodites, 8.3% contained only female gametes, and 31.1% contained only male gametes, and the remaining 7.2% were indeterminate. Across all treatments, gonad sex (collapsed for comparison) differed significantly among cohorts ($\chi^2=55.8$, $p=1.0e-4$). Fifty percent of all O-1 oysters sampled were female or hermaphroditic-primarily female (HPF), while 33%, 24% and 11% of D, F, and O-2 were female or HPF. Male or hermaphroditic-primarily male oysters comprised 29%, 48%, 59% and 69% of O-1, D, F, and O-2 cohorts, respectively.



Supplementary Figure 2: Gonad sex for each cohort, after temperature treatment but before pCO₂ treatment (“Pre”), and after 52 days in high pCO₂ (3045±488 μatm, n=39, “High”), and ambient pCO₂ (7.82±0.02, n=39, “Ambient”), separated by temperature treatment (6°C and 10°C).

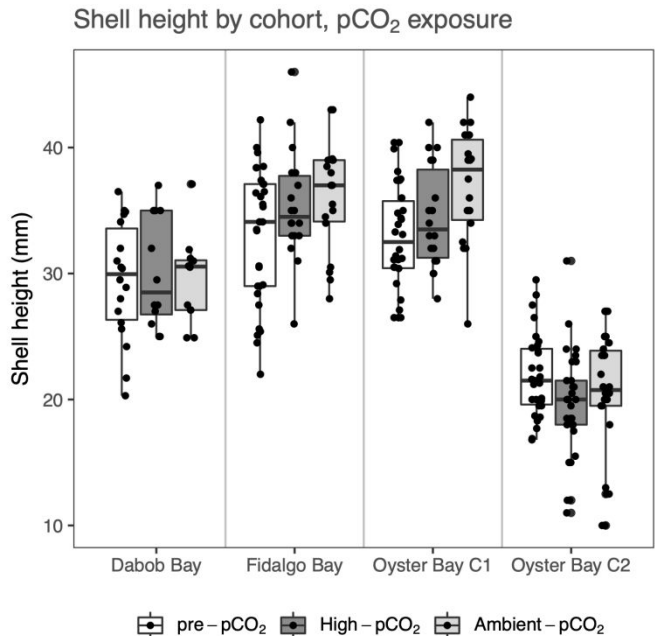


Supplementary Figure 3: Gonad stage of the dominant sex for each cohort, after temperature treatment but before pCO₂ treatment (“Pre”), and after 52 days in high pCO₂ (3045±488 µatm, n=39, “High”), and ambient pCO₂ (7.82±0.02, n=39, “Ambient”), separated by temperature treatment (6°C and 10°C).

Adult shell height

Oysters sampled for histology were also measured for shell height using digital calipers (mm), defined as the maximum distance from the umbo along the dorsal/ventral axis. Shell height was compared between treatments prior to and after pCO₂ exposure using two-way Analysis of Variance (ANOVA) for each cohort. Shell height was also compared among cohort using one-way ANOVA, excluding the younger O-2 cohort due to their smaller initial size.

Prior to pCO₂ treatments, adult shell height did not vary between temperatures treatments, but did among F1 cohorts, with D smaller than F (p=0.043). After pCO₂ treatment, D was smaller than both F (p=3.4e-6) and O-1 (p=3.5e-6). The O-1 cohort increased in size (p=0.019) in ambient pCO₂, but not in high pCO₂. No size differences among pre-pCO₂ and post-pCO₂ treatments were observed in the F, D or O-2 cohorts.



Supplementary Figure 1: Adult shell height for each cohort, after temperature treatment but before pCO₂ treatment (“Pre-pCO₂”), and after 52 days in High-pCO₂ (3045±488 µatm, n=39), and Ambient-pCO₂ (7.82±0.02, n=39).

Larval collection differences among cohorts

Total larvae collected differed by cohort ($F(3,8)=15.3$, $p=0.001$). O-1 produced significantly more total larvae than F and O-2 ($p=0.0094$, $p=0.0014$, respectively), and D produced more total larvae compared to O-2 ($p=0.022$). Total larvae released by O-1, F, D, and O-2 was 10.1M, 3.6M, 2.7M and 2.1M, respectively. The same patterns were observed in average daily larvae released by cohort ($F(3,20)=8.9$, $p=0.0009$). Date of first larval release differed by cohort ($F(3,8)=15.1$, $p=0.0012$). Oyster Bay cohorts (O-1 and O-2) released larvae 9.9 days earlier than F and D cohorts on average. Larval pulse frequency differed by cohort ($F(3,8)=9.8$, $p=0.0046$). On average, O-1, O-2, F, and D released larvae 6.4 ± 2.3 , 8.0 ± 2.9 , 3.8 ± 1.9 , and 2.8 ± 1.0 days, respectively. The O-1 cohort released larvae more frequently than F ($p=0.017$), and O-2 more frequently than both F and D ($p=0.0066$, $p=0.043$, respectively).

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Supplementary Table 5: Timing and magnitude of larval production in 4 *Ostrea lurida* cohorts previously exposed to different winter temperatures (6°C and 10°C), then pCO₂ treatments (“Amb.” is Ambient=841±85 µatm, pH 7.82±0.02, High=3045±488 µatm). Fidalgo Bay, Dabob Bay, and Oyster Bay are previously identified as genetically distinct populations (Jake E. Heare et al., 2017; J. Emerson Heare et al., 2018a). Two Oyster Bay cohorts were used (O-1, O-2), with O-2 being the offspring of O-1 and likely all siblings. For each metric, total (“Tot.”) or mean (“Ave.”) of all cohorts combined for each treatment is shown.

Cohort		Fidalgo Bay (2 reps)		Dabob Bay		Oyster Bay - F1 (2 reps)		Oyster Bay - F2		All cohorts combined	
pCO ₂ treatment		Amb. pCO ₂	High pCO ₂	Amb. pCO ₂	High pCO ₂	Amb. pCO ₂	High pCO ₂	Amb. pCO ₂	High pCO ₂	Amb. pCO ₂	High pCO ₂
No. of broodstock	6°C	15/14	14/15	14	15	15/16	17/17	117	126	Tot: 191	Tot: 204
	10°C	15/14	14/14	9	16	17/17	15/15	115	111	Tot: 177	Tot: 185
Date of first release	6°C	142/139	156/145	163	133	134/137	140/141	139	137	Ave: 142	Ave: 142
	10°C	145/137	144/141	144	146	135/134	134/137	131	131	Ave: 138	Ave: 139
Date of max release	6°C	149/146	161/145	163	168	135/156	141/165	154	143	Ave: 151	Ave: 154
	10°C	145/137	144/144	151	147	135/152	151/137	140	143	Ave: 143	Ave: 144
Date of last release	6°C	173/149	173/191	187	173	170/170	168/168	173	161	Ave: 170	Ave: 172
	10°C	158/168	184/191	182	175	191/187	175/173	166	170	Ave: 175	Ave: 178
Ave. daily	6°C	21/38	99/127	53	107	139/114	103/175	58	43	Ave: 79	Ave: 110

larvae released (x10 ³)	10°C	139/84	58/56	117	101	192/107	133/111	36	57	Ave: 108	Ave: 88
Total larvae released (x10 ³)	6°C	127/150	591/892	105	959	697/1,482	719/1,397	518	389	Tot: 3.08M	Tot: 4.95M
	10°C	695/421	345/393	939	705	1,918/1,502	933/1,441	466	689	Tot: 5.9M	Tot: 4.5M
Total larvae released per broodstock (x10 ³)	6°C	2.4/3.0	11.7/16.5	2.5	21.3	12.9/25.7	11.7/22.8	2.0	1.4	Ave: 6.6	Ave: 13.5
	10°C	1.3/8.4	6.8/7.8	34.8	14.7	31.3/24.6	17.3/26.7	1.8	2.8	Ave: 18.8	Ave: 11.7
Maximum release (x10 ³)	6°C	111/140	247/308	105	356	462/484	250/809	133	131	Ave: 239	Ave: 350
	10°C	248/246	298/186	437	268	555/407	378/379	108	241	Ave: 333	Ave: 292
No. big release days (>10k)	6°C	2/1	4/4	1	4	3/8	5/5	6	5	Tot: 21	Tot: 27
	10°C	3/3	2/3	5	5	7/7	5/10	11	10	Tot: 36	Tot: 35

Review Only

Larval rearing methods

Larvae collected between May 19 and July 6 were separated by treatment and cohort and reared over 67 days from May 19 to July 25. For all culture tanks, seawater was heated to 18°C in a common 1,610-L recirculating reservoir (1610 L) using Aqua Logic digital temperature controllers (TR115SN), dosed with live algae cocktail via an Iwaki metering pump to achieve 100,000 cells/mL, and distributed to culture tanks. Larvae were grown in two connected 19-L flow-through tanks (19-L; 8-L/hr) with aerated, filtered seawater (1 µm) at 18°C. The two-tank larval system was used to cull dead larvae: water flowed from one 19-L tank where larvae were added but non-swimming larvae would remain (“mortality tank”) to the next (“live tank”), carrying live, swimming larvae which were then contained on a 100 µm screen. Twice weekly, live larval tanks were screened into three size classes: 100 µm < X < 180 µm (“100 µm”), 180 µm < X < 224 µm (“180 µm”), >224 µm (“224 µm”, which is when *O. lurida* larvae are near metamorphosis). Each size class was subsampled and counted, then the 100 µm and 180 µm classes returned to larval tanks. The number of live larvae returned to culture tanks informed stocking of newly released larvae. To maximize genetic diversity of offspring, newly spawned larvae ($\leq 50,000$) were added to culture tanks continuously to a maximum stocking density of 200,000 larvae (~10 larva/mL) (PSRF pers. communication & FAO manual). The contents in the mortality buckets were collected during biweekly screenings on a 100 µm screen to count live and dead oysters, but live were not kept.

During the twice weekly screening days, larvae that were larger than 224 µm were moved to downwelling setting silos, separated by cohort, temperature and pCO₂ treatment. Setting tanks were 180 µm silos with 18°C filtered seawater (1 µm) dosed with live algae, which then flowed into each silo from 8-L/hr irrigation drippers. Pacific oyster shell fragments (224 - 450 µm) were sprinkled into each silo to cover the surface to provide a settlement substrate. Silos were cleaned with freshwater (18°C) several times per week. Live, metamorphosed oysters were counted on August 12 for survival rate from 224 µm to post-metamorphosis (“post-set”), then transferred to 450 µm silos with ~17°C upwelling filtered seawater (5 µm) to continue rearing. Oysters were fed live algae using a gravity algae header tank, and rinsed 1-2 times per week with freshwater. On October 4, when oysters were between 13-20 weeks old, all groups were moved to screen pouches separated by cohort x temperature x pCO₂, affixed to the inside of shellfish cages, and hung in Clam Bay until June 2018.

Supplementary Table 6: Tank densities during offspring rearing.											
		<i>Fidalgo Bay (2 replicates)</i>		<i>Dabob Bay</i>		<i>Oyster Bay - F1 (2 replicates)</i>		<i>Oyster Bay - F2</i>		<i>All</i>	
		Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂
Cumulative larvae stocked	6°C	169,475	353,916	65,667	255,371	477,403	532,417	353,769	179,717	1,066,314	1,321,421
	10°C	319,277	271,016	283,858	310,875	937,920	660,451	324,166	237,790	1,865,221	1,480,132
Mean, larvae density (in 20L tank)	6°C	29,148 ± 33,256	54,265 ± 44,431	22,000 ± 22,036	43,293 ± 43,168	50,260 ± 51,338	58,662 ± 64,276	47,231 ± 38,553	31,877 ± 30,341	39,270 ± 40,579	46,777 ± 47,718
	10°C	40,150 ± 47,430	29,486 ± 30,781	49,584 ± 33,070	56,081 ± 49,214	88,962 ± 71,752	74,562 ± 72,312	53,588 ± 50,765	32,423 ± 41,853	60,478 ± 5,7472	48,774 ± 54,447
Median larvae density	6°C	18,013	42,783	19,117	26,160	33,797	32,608	45,667	20,800	26,397	28,192
	10°C	16,000	20,650	50,317	45,833	83,577	48,800	29,833	7,877	50,317	34,935
Total eyed larvae	6°C	11,119	11,780	2,496	10,686	11,931	6,029	22,186	9,735	47,732	38,230
	10°C	3,737	2,978	13,862	19,815	59,929 (split)	10,670	13,828	2,910	91,356	36,373
Total post set (singles)	6°C	1,503 (split)	670	501	834	124	192	356	334	2,484	2,030
	10°C	626	75	1311	1091	52	34	246	113	2,235	1,313
Juvenile shell height (mm)	6°C	9.0±2.7	8.4±3.5	6.5±1.9	4.9±2.3	11.2±3.5	11.0±3.4	11.0±3.7	7.5±3.0		
	10°C	9.7±3.0	12.3±5.4	7.0±2.3	6.6±2.9	11.0±3.8	12.2±4.2	10.7±3.7	13.4±4.4		
Juveniles deployed	6°C	240	257	240	240	85	77	122	90	677	664
Juveniles survived	6°C	60	159	72	81	32	45	22	4	186	289

Larval survival estimates

Larval survival was estimated from both twice-weekly larval counts and cumulative survival counts. Percent survival between biweekly larval counts was calculated by summing the number of live larvae in all size classes (100 μm , 180 μm , 224 μm), dividing by the number of live larvae restocked after the previous count, plus all new larvae added since. Cumulative percent survival from newly released larvae (“new larvae”) to the near-metamorphosis stage (“eyed larvae”), and to post-metamorphosis (“post-set”) were compared between treatments based on total number of new larvae stocked in culture tanks and eyed larvae in setting tanks over the larval rearing period. During larval rearing, culture tank densities were capped at 200,000 larvae (~10 larvae/mL), but ranged during the 67 day larval rearing period due to varying mortality and larval release timing. Daily tank densities were estimated from twice-weekly larval counts and number of new larvae added, then compared between temperature x pCO₂ treatments using a Kruskal-Wallis Test.

Biweekly larval survival, cumulative survival from new to eyed larvae, and survival from eyed larvae to post-set were compared among cohort x temperature x pCO₂ treatments using ANCOVA on fitted linear regression models. For biweekly percent survival, square-root arcsine transformation was applied, and biweekly tank density was included as a random effect. For cumulative survival models, mean stocking density and total larvae stocked in culture tanks were examined as candidate random effects with Pearson’s correlation using `pairs` and `cor`. For post-set survival, cumulative eyed larvae stocked in setting tanks and percent survival to eyed larvae stage were also tested and survival data was log-transformed. Tank density factors that correlated significantly with cumulative survival were considered as random effects in full regression models alongside cohort, temperature and pCO₂. All models were optimized using stepwise deletion and selected based on AIC value, adjusted R-squared, and F-statistic.

Larval survival results

Larval survival between biweekly counts did not differ by pCO₂ or temperature, but did differ by cohort ($F(3,230)=5.73$, $p=8.5\text{e-}4$). Pairwise tests indicate that O-1 survival was significantly lower than D ($p=3.8\text{e-}4$), O-2 ($p=5.4\text{e-}4$), and F ($p=0.019$). Mean biweekly survival of D, F, O-2, and O-1 cohorts was $62\pm22\%$, $59\pm24\%$, $55\pm24\%$, and $49\pm28\%$, respectively. Cumulative survival from new- to eyed-larvae was low across all treatments, and did not differ by parental temperature treatment ($F(1,14)=2.3$, $p=0.15$), parental pCO₂ ($F(1,14)=1.9$, $p=0.19$), or cohort ($F(3,12)=1.4$, $p=0.29$) (Table 3). Cumulative survival from eyed larvae to post-set) ranged from 0.2% to 26.5% and differed by cohort ($F(3,11)=3.8$, $p=0.04$). Pairwise tests revealed that this was influenced by low survival in the O-1 group and significance was not strong after removing O-1 ($F(2,9)=4.1$, $p=0.06$). No survival differences through metamorphosis were detected between pCO₂ or temperature treatments.

Tank density prior to each biweekly screening was a significant factor influencing survival between bi-weekly counts ($F(1,230)=10.4$, $p=0.0015$) and therefore was included as a random effect in the biweekly survival regression model. Mean stocking densities across the 67-day rearing period in O-1, D, O-2, and F were $76,500\pm71,100$, $54,400\pm42,400$, $47,000\pm46,200$, and $43,500\pm42,700$, respectively. No random effects were retained in the cumulative survival from new- to eyed-larvae model. Total larvae stocked in larval culture tanks correlated with survival from eyed-larvae to post-set (i.e. through metamorphosis), and therefore was included as a random effect in the post-set survival model.

The number of days between first and last larval collection, and first and last eyed larvae varied by cohort, although this was not significant. Across treatments, eyed larvae were present soonest in F (14.5 ± 2.5 days), followed by O-1 (16.5 ± 1.75 days), O-2 (17.25 ± 1.25 days), and lastly D (18.25 ± 3 days) ($F(3,12)=2.0$, $p=0.16$). The number of days between stocking the last batch of newly released larvae, and collecting the last eyed larvae were 22 ± 5.8 , 23.25 ± 7.4 , 29.5 ± 4.7 , and 32 ± 4.8 for O-1, F, D, and O-2, respectively.

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Supplementary Table 7: Larval survival estimates by parental treatment and cohort.											
Larval survival, by treatment and cohort											
		Fidalgo Bay		Dabob Bay		Oyster Bay - F1		Oyster Bay - F2		All cohorts	
		Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂	Amb pCO ₂	High pCO ₂
Average biweekly larval survival	6°C	56±25%	62±26%	69±18%	49±21%	44±23%	52±27%	60±24%	59±28%	56±24%	56±26%
	10°C	50±18%	51±26%	58±25%	70±18%	49±30%	46±29%	63±25%	55±21%	55±25%	56±25%
Cumulative survival to eyed larvae	6°C	8.5%	3.0%	5.1%	4.7%	2.7%	1.2%	6.7%	5.7%	5.7±2%	3.6±2%
	10°C	1.3%	1.4%	5.2%	6.6%	4.2%	0.7%	4.4%	1.0%	3.8±1%	2.4±3%
Cumulative survival, eyed larvae to post set	6°C	13.8%	5.9%	26.5%	9.3%	1.1%	3.6%	1.7%	3.5%	10.8 ±10%	5.6 ±2.7%
	10°C	18.5%	2.7%	9.7%	6.0%	0.2%	0.7%	1.9%	5.8%	7.6±8%	3.8±3%