

WILEY

ECOLOGICAL APPLICATIONS

**Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations**

Journal:	<i>Ecological Applications</i>
Manuscript ID:	EAP19-0502.R1
Wiley - Manuscript type:	Articles
Date Submitted by the Author:	07-Oct-2019
Complete List of Authors:	Spencer, Laura; University of Washington, School of Aquatic and Fishery Sciences Venkataraman, Yaamini; University of Washington, School of Aquatic and Fishery Sciences Crim, Ryan; Puget Sound Restoration Fund Ryan, Stuart; Puget Sound Restoration Fund Horwith, Micah; Washington State Department of Natural Resources Roberts, Steven; University of Washington College of the Environment,
Substantive Area:	Reproductive Strategies < Population Dynamics and Life History < Population Ecology < Substantive Area, Variation < Population Genetics and Breeding Systems < Population Ecology < Substantive Area, Inheritance/Heritability < Population Genetics and Breeding Systems < Population Ecology < Substantive Area, Physiological Ecology < Substantive Area, Metapopulations < Population Dynamics and Life History < Population Ecology < Substantive Area
Organism:	Bivalves < Molluscs < Invertebrates < Animals
Habitat:	Estuarine < Marine < Aquatic Habitat < Habitat, Intertidal/Tidal/Coastal < Marine < Aquatic Habitat < Habitat
Geographic Area:	Northwest US (ID, MT, OR, WA, WY) < United States < North America < Geographic Area
Additional Keywords:	acidification, pH, reproduction, winter, phenology, intergenerational, transgenerational, climate change, <i>Ostrea lurida</i> , warming
Abstract:	Predicting how populations will respond to ocean change across generations is critical to effective conservation of marine species. One emerging factor is the influence of parental exposures on offspring phenotype, known as intergenerational carryover effects. Parental exposure may deliver beneficial or detrimental characteristics to offspring that can influence larval recruitment patterns, thus shaping how populations and community structure respond to ocean change. Impacts of adult exposure to elevated winter temperature and pCO <sub>2</sub> on reproduction and offspring viability were examined in the Olympia oyster ( <i>Ostrea lurida</i> ) using three populations of adult, hatchery-reared <i>O. lurida</i> , plus an additional cohort spawned from one of the populations.

Oysters were sequentially exposed to elevated temperature (+4°C, at 10°C), followed by elevated pCO<sub>2</sub> (+2204 µatm, at 3045 µatm) during winter months. Male gametes were more developed after elevated temperature exposure and less developed after high pCO<sub>2</sub> 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<sub>2</sub> exposure. Those exposed to elevated winter temperature as a sole treatment released more larvae on a daily basis, but when also exposed to high pCO<sub>2</sub> there was no effect. These combined results indicate that elevated winter temperature accelerates *O. lurida* spermatogenesis, resulting in earlier larval release and increased production, with elevated pCO<sub>2</sub> exposure negating effects of elevated temperature. Altered recruitment patterns may therefore follow warmer winters due to precocious spawning, but these effects may be masked by coincidental high pCO<sub>2</sub>. Offspring were reared in common conditions for one year, then deployed for three months in four estuarine bays with distinct environmental conditions. Offspring of parents exposed to elevated pCO<sub>2</sub> had higher survival rates in two of the four bays. This carryover effect demonstrates that parental conditions can have substantial ecologically relevant impacts that should be considered when predicting impacts of environmental change. Furthermore, Olympia oysters may be more resilient in certain environments when progenitors are pre-conditioned in stressful conditions. Combined with other recent studies, our work suggests that the Olympia may be more equipped than other oysters for the challenge of a changing ocean.

SCHOLARONE™  
Manuscripts

**Responses to reviewer comments for “Carry-over effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations”, submitted to Ecological Applications.**

**REVIEWER 1 COMMENTS:**

---

**COMMENT:** For Ecological Applications, I would suggest that the study should be placed in a somewhat broader conceptual framework. The very start of the Introduction could begin with a few sentences about understanding population responses to climate change across generations or about the importance of realistic temporal exposure to climate-related stressors, before diving into marine bivalves. Likewise, the first sentence of the Abstract could open with a broader statement about the field of inquiry, to draw in readers from other systems. Finally, either the first or last paragraph of the Discussion could again zoom out to a broad level, discussing general implications of the work that would apply to any system (importance of realistic timing, of examining population variation, context-dependence, etc.) and citing non-bivalve papers.

**RESPONSE:** The Abstract, Introduction, and Discussion have been revised, and include broader statements and implications as suggested.

---

**COMMENT:** The Introduction is generally well-written, but switches a bit between general bivalve information to information specifically relevant to the current study (Puget Sound conditions, Olympia oyster biology). A somewhat clearer organization might be helpful, for instance having a first short paragraph be general/conceptual, then following with a few paragraphs on marine bivalves facing changing ocean conditions, then following with a paragraph or two specific to Olympia oysters, and finishing with the specific goals and predictions of this study.

**RESPONSE:**

Based on this and the previous comment, the introduction now begins more broadly by introducing intergenerational carryover effects across marine phyla, then focuses on evidence in bivalves. I then provide background on key factors in our study: the importance of testing exposures using an ecologically realistic timeframe, and monitoring reproduction. I then introduce the Olympia oyster, recruitment issues that may be influenced by winter conditions and parental carryover (as suggested by another reviewer), intraspecific variation in the Olympia oyster that influence experimental results, and finally the study details and predictions.

---

**COMMENT:** For the section on gametogenesis, the rationale for the assessment needs to be clarified. It is not clear whether the evaluations pertain to individual fitness or population level reproductive output. Text is needed to explain why staging the gonads is a good indicator, and why the particular snapshots in time chosen are relevant predictors of

**reproduction.** Likewise, in the Results, the significance of Figure 4 and 5 needs to be more clearly explained, in terms of indicators of fitness or population growth rate or whatever.

**RESPONSE:** Thank you for this suggestion. The below was added to the Methods to explain why we sampled gonad after each treatment. I hope this will provide needed context to readers, particularly ecologists that are not familiar with gametogenic stages and cycles. Additionally, Figure 4 and 5 captions were expanded to highlight observed differences.

#### **"Adult reproductive development**

A subset of oysters were sampled for gamete stage and dominant sex immediately before and after pCO<sub>2</sub> treatments (Figure 2) to capture developmental differences among treatments. Puget Sound *O. lurida* reportedly enter reproductive quiescence and resorb residual gametes when temperatures are below 12.5°C (Hopkins 1936, 1937), however recent evidence of low-temperature brooding (10.5°C, Barber et al. 2016) suggests that reproductive activity may occur during warm winters. Therefore, gonad tissue was sampled to estimate whether residual gametes were resorbed or developed during winter treatments, whether temperature and pCO<sub>2</sub> influenced winter activity, if male and female gametes responded similarly, and if effects correspond with fecundity."

---

**COMMENT:** The community of researchers studying Olympia oysters is rather small, such that only a few papers a year are published. For this reason, it was surprising that a few highly relevant papers were not cited. **The following papers should be cited, and moreover, their findings should be integrated with the results of the current study in the Discussion.**

**RESPONSE:** Thank you for providing these resources. Admittedly some of these references were omitted during the co-author editing process, but are indeed vital to provide a comprehensive picture of how the Olympia oyster responds to the environment. The references have been incorporated as follows:

#### **Barber et al. 2016 in Methods:**

"Puget Sound *O. lurida* reportedly enter reproductive quiescence and resorb residual gametes when temperatures are below 12.5°C (Hopkins 1936, 1937), however recent evidence of low-temperature brooding (10.5°C, Barber et al. 2016) suggests that reproductive activity may occur during warm winters."

#### **Bible et al. 2017 in Discussion:**

"Despite experimental differences (e.g. sequential vs. simultaneous exposures) which can influence outcomes (Bible et al. 2017), both Parker et al. (2018) and the present study indicate that high pCO<sub>2</sub> slows gametogenesis, elevated temperature accelerates it, and these two environmental drivers act antagonistically on gonad development if occurring in the same reproductive season."

**Cheng et al 2015** in Discussion:

"An important factor not included in either study is ecologically relevant variability. Temperature and pCO<sub>2</sub> oscillations, driven by tides and diurnal photosynthesis, could offer daily refuge or expose Olympia oysters to dynamic changes, and altering how combined stressors interact (Cheng et al. 2015)."

**Moore et al. 2016** in Discussion:

"Future research should examine *O. lurida* sexual development during the initial switch from male to female, which can occur the first winter after settlement (Moore et al., 2016), and across a range of pCO<sub>2</sub> and temperatures to determine conditions in which gametogenesis and sex determination are affected."

---

**COMMENT:** Line 13: for a broad journal such as Ecological Applications, I'd suggest that the first sentence of the Abstract should be more general and conceptual, about the area of inquiry, not just Olympia oysters.

**RESPONSE:** Abstract now begins with a broad statement:

"Predicting how populations will respond to oceans change across generations is critical to effective conservation of marine species. One emerging factor is the influence of parental exposures on offspring phenotype, known as intergenerational carryover effects. Parental exposure may deliver beneficial or detrimental characteristics to offspring to influence larval recruitment patterns, which will shape how populations, and thus community structure, respond to ocean change."

---

**COMMENT:** Line 19: towards the end of the abstract, it would seem worth highlighting that Olys may actually do better under projected climate change, or at least that for the scenarios tested here, they seem like they will not do worse

**RESPONSE:** While I think it's premature to argue conclusively that Olys will benefit from changing conditions, it is heartening that multiple studies have found neutral or positive responses to climate conditions, particularly compared to other species (e.g. Pacific). I included potential implications for *Ostrea lurida* as a "winner" in the abstract and conclusion.

**Abstract:**

"Furthermore, depending on the offspring environment, Olympia oysters may be more resilient when progenitors are pre-conditioned in similarly stressful conditions, which combined with other recent studies suggests that the Olympia may be more equipped than other oysters for the challenge of a changing ocean."

**Conclusion:**

"Combined with previous reports of resilience to environmental stressors (Waldbusser et al 2016; Cheng et al. 2017) and intraspecific variability (Bible, Evans & Sanford, 2019; Maynard, Bible, Pespeni, Sanford, & Evans, 2018; Silliman, Bowyer, & Roberts, 2018; Heare, Blake, Davis, Vadopalas, & Roberts, 2017), the Olympia oyster may be more capable than other marine bivalve species to withstand and adapt to unprecedented ocean change. Furthermore, conserving and restoring *O. lurida* in a variety of settings – including hypoxic, warmer, and less alkaline areas – could increase the probability that future populations are equipped for challenging conditions through selection or intergenerational carryover."

---

**COMMENT:** Line 36: "the focus" is misleading, as there are of course many focus areas for such research; rephrase to indicate that this is one area of interest, not the only one

**RESPONSE:** Thank you, I agree. It now reads, "one area of interest is whether"

---

**COMMENT:** Line 56: "are promising" is unclear – is this a compliment to the quality of the studies, or the conservation implications of the results?

**RESPONSE:** It now reads, "A foundational series of studies on the Sydney rock oyster (*Saccostrea glomerata*) provide strong evidence for intergenerational carryover effects in estuarine bivalves."

---

**COMMENT:** Line 66: change to "only one study" (or delete the "to our knowledge")

**RESPONSE:** Changed to "only one study".

---

**COMMENT:** Line 97: this statement isn't exactly true, as other oysters occur in Mexico

**RESPONSE:** I appreciate you mentioning this. At conferences I routinely see speakers (including myself) begin Oly presentations with "the only native oyster species on the N. American Pacific Coast". I will be more vigilant moving forward!

---

**COMMENT:** Line 98: remove apostrophe from 1900's

**RESPONSE:** Removed.

---

**COMMENT:** Line 120: does “this study” refer to the Hettinger in the previous sentence, or to your own study? If the latter, move this information to the following paragraph where you introduce your study.

**RESPONSE:** “This study” referred to our study. That sentence was merged with the 1st sentence in the final Introduction paragraph.

---

**COMMENT:** Line 124: add a name to this unpublished observation (guessing it’s from author Ryan Crim?)

**RESPONSE:** Correct, observations were from Ryan Crim; his name was added.

---

**COMMENT:** Line 156, 168, 172, 175, etc: there are a lot of details about make and model of equipment used in the Methods that could be relegated to the Supplement. This is perhaps a call for the editor to make; it is a matter of taste whether this sort of detail is desirable in the main text or not.

**RESPONSE:** The make and model information for all instruments has been moved to the supplementary materials. The following statement was added to the end of the Methods section:

“Make and model details for instruments used during treatments and field deployments are available in the Supplementary Materials.”

---

**COMMENT:** Line 190 ff: it would be helpful to add some overview sentences about the rationale/approach for the assessment of reproduction. Since the same individuals were not tracked over time, and since individuals in a population are not synchronous (Moore et al. 2016) it seems impossible to tell if a needed “quiescent” period was absent, for instance. It also seems unclear how the particular snapshot in time for making the assessment was chosen, and why that time was particularly informative. Thus, **you need to make a clearer case for why the results are representative or predictive of individual oyster fitness or overall oyster population output or whatever you had in mind.**

**RESPONSE:** Language was added to the Methods section entitled “Adult reproductive development”, and to Figure 4 and 5 captions, as described above.

---

**COMMENT:** Line 221: in my version, symbol here that presumably represented alpha came out as box (Mac/PC issues?)

**RESPONSE:** Yes, it must be a conversion issue. I saved to PDF this time before uploading; hopefully that solves the problem.

---

**COMMENT:** Line 225 (and 237): instead of “April 11th”, give full date

**RESPONSE:** Now reads, “April 11, 2017”.

---

**COMMENT:** Line 227 & 756: what is spawning “volitionally”?

**RESPONSE:** Explanation added: “Oysters spawned in the hatchery for 90 days volitionally, i.e. naturally releasing gametes without chemical or physical manipulation.”

---

**COMMENT:** Line 232: “viviparous spermcaster” is a bit confusing, maybe just explain they release sperm but have internal fertilization and release larvae following a brooding period

**RESPONSE:** Thank you, I have incorporated your language:  
“Olympia oysters release sperm, but have internal fertilization and release veliger larvae following a ~2 week brooding period. Therefore, larval production was assessed by collecting veliger larvae upon maternal release.”

---

**COMMENT:** Line 289 & 298: by “differences in sex”, do you mean “differences in sex ratio”?

**RESPONSE:** Correct, both lines now read “sex ratio”.

---

**COMMENT:** Line 310-314: clarify why # of larvae produced per day differed by temperature, but total larvae produced did not; does not seem to follow?

**RESPONSE:** Thank you for catching this lack of clarity. The average larvae metric does not include days where zero larvae were collected. This metric may therefore be related to female brood size (more larvae released at once), or synchronous larval release (several females releasing larvae in one day). Total larvae produced over the 90-day collection period refers to the sum of all larvae collected across all days, and tended to differ among treatments, but it was not significant ( $p=0.06$ ).

To add clarity, I adjusted language in the below sections. In addition, I altered Figure 6 to show the average daily pulse before\_normalizing by broodstock size and number in an effort to make the plot more intuitive and informative.

**Abstract:** “Those exposed to elevated winter temperature as a sole treatment released more larvae on a daily basis, but when oysters were also exposed to high pCO<sub>2</sub> there was no effect.”

**Methods:** “...total larvae released across the 90-day period, average number of larvae collected on a daily basis (excluding days where no larvae were released), ...”

**Results:** “Adults exposed to 10°C produced more larvae on a daily basis (excluding days where no larvae were released) than those exposed to 6°C in ambient pCO<sub>2</sub>-exposed oysters ( $p=0.040$ ).”

**Figure 6:** “Left: average number of larvae collected on a daily basis (excluding days where no larvae were released). Daily pulses of larvae were larger in 10°C than 6°C, but only in oysters exposed to ambient pCO<sub>2</sub>. For statistical analysis, average daily release was normalized by number of oysters \* average oyster height (cm) (data shown is not normalized).”

---

**COMMENT:** Line 325: those seem like very low female numbers compared to the entire number of oysters used? Especially since you indicate most oysters had active gonads of one sort or another?

**RESPONSE:** The number of oysters that had active female gonad was indeed high, but the oocyte stage varied, so may have needed additional time to develop to ripe ova. Additionally, many oysters were hermaphroditic so could have spawned as male during the experiment despite also containing late-stage oocytes. Had we continued collecting they may have subsequently spawned as female.

Furthermore, the estimates in the manuscript are based on a previous study’s report of 215,000 larvae / female at 35 mm in the wild, and was included to provide a ballpark for the number of families potentially contributing to offspring. Some of our oysters were smaller than 35 mm, and since fecundity is positively related to size the number of contributing females is likely higher than what I listed (this caveat is described in the manuscript).

---

**COMMENT:** Line 337ff: instead of chi the symbol shows up as a box in my version; conversion error when pdf was made by journal, presumably

**RESPONSE:** Noted, thank you.

---

**COMMENT:** Line 355: not clear why mortality should affect this measure? Explain.

**RESPONSE:** Thank you for raising this issue. I had questioned the size/mass data due to varying mortality in bags resulting in varying stocking density, which based on prior studies can cause stocking-density related growth differences. However, upon seeing your question, I

reviewed the size, growth, and survival/stocking density data, and found that there was no relationship. Therefore, I am now confident in the growth data, and I have removed the sentence previously at line 355. Furthermore, there are differences in mass among cohorts and bay (not parental pCO<sub>2</sub>), which are now included in the results, but do not alter our conclusions.

---

**COMMENT:** Line 358: not clear why crashing populations would have greater sensitivity

**RESPONSE:** The intention was to highlight that the Olympia oyster as a species is already vulnerable due to major population crashes. I see your point, as crashes could result in highly fit individuals (for instance). This language has been removed.

---

**COMMENT:** Line 361: “leveraged” does not seem like right word here, “examined”, maybe?

**RESPONSE:** Replaced with “examined”.

---

**COMMENT:** Line 374: hard to imagine sperm are limiting reproduction within hatchery conditions?

**RESPONSE:** Mature sperm may have been more limiting than is usual in the hatchery, as each spawning tank only contained a couple dozen oysters. This statement was removed, however, as factors other than those measured could have certainly influenced larval production (e.g. fertilization rate, percentage of mature ova actually expelled).

---

**COMMENT:** Line 380: “this study” is ambiguous: yours or the Macoma one?

**RESPONSE:** Replaced with “the present study”.

---

**COMMENT:** Line 385: replace “things” with “factors” or some other word

**RESPONSE:** Replaced with “factors” as suggested.

---

**COMMENT:** Line 439: add “with” after “contrast”

**RESPONSE:** Added “with” as suggested.

---

**COMMENT:** Line 440: “they” doesn’t make sense here – maybe combine with previous sentence

**RESPONSE:** The two sentences are now one: “Our results contrast with a similar study that exposed *C. gigas* oysters to high pCO<sub>2</sub> during the winter, and found fewer hatched larvae 18 hours post-fertilization from exposed females, with no discernable paternal effect (Venkataraman et al., 2019).”

---

**COMMENT:** Line 468: is “plasticity” the right word here?

**RESPONSE:** The term “transgenerational plasticity (TGP)” is commonly used to refer to cross-generational carryover effects. Because we tested only one generation, I instead use the term “intergenerational” in lieu of transgenerational to avoid misinterpretation. Some references on TGP:

- Galloway, L. F., & Etterson, J. R. (2007). Transgenerational plasticity is adaptive in the wild. *Science*, 318(5853), 1134–1136.
- Ross, P. M., Parker, L., & Byrne, M. (2016). Transgenerational responses of molluscs and echinoderms to changing ocean conditions. *ICES Journal of Marine Science: Journal Du Conseil*, 73(3), 537–549.
- Gavery MR, Roberts SB. 2017. Epigenetic considerations in aquaculture. *PeerJ* 5:e4147 <https://doi.org/10.7717/peerj.4147>

---

**COMMENT:** Line 470-474: there is some jumping around between descriptors that apply to individuals vs. descriptors that apply to populations – stick to one level of organization throughout these recommendations

**RESPONSE:** Simplified: “1) source population; 2) environmental history (within-lifetime carryover effects); and 3) ancestral environmental history (inter- and transgenerational carryover effects).”

---

**COMMENT:** Line 485: odd use of exclamation mark

**RESPONSE:** Replaced with period.

---

**COMMENT:** Table 1: indicate the years/months when measurements occurred, and define measurement units for chlorophyll

**RESPONSE:** Added both items.

---

**COMMENT:** Tables 2-3: there is so much information here that it is hard to absorb. Perhaps it'd be possible to use color coding, e.g. conditional formatting in Excel to make the high values pop out, so the reader can quickly see which treatments yielded the highest levels for each indicator?

**RESPONSE:** Tables have been simplified. As suggested, I also color-coded in grayscale to improve readability (which was a great idea!), but was informed that the EA guidelines do not allow for any shading in tables. Hopefully the simplifications have improved readability.

---

**COMMENT:** Fig 2: extremely clear and helpful overview!

**RESPONSE:** Fantastic, glad it was so helpful!

---

**COMMENT:** Fig 3: suggest moving this to Supplement, as most readers are ecologists and won't be able to make heads or tails of this. Also might be helpful to add some arrows, asterisks, etc. to point out key identifying features for some/all of the photos

**RESPONSE:** Yes, this is smart, especially given the number of plots in the manuscript. This figure has been moved to the Supplementary for those interested in seeing examples of Olympia oyster gonad tissue.

---

**COMMENT:** Fig 6: font size is too small

**RESPONSE:** Font size was increased.

---

**REVIEWER 2 COMMENTS:**

**COMMENT:** Given the target journal, one area where the study could improve involves establishing a linkage to a particular ecological application. Can the authors better explain how this study fits a particular ecological problem, issue, or policy decision... other than trying to predict the effects of climate change?

**RESPONSE:** Thank you for the suggestion, and for providing these excellent references. The ecological questions that our study investigates, and implications of our results, are indeed critical to communicate to EA readers. One key area is how our study relates to recruitment patterns. The inter-annual recruitment variability and frequent failures reported in Kimbro et al. (2019) and Wasson et al. (2016) are very interesting, and suggest that recruitment patterns are in part governed by local environmental conditions, larval retention, and possibly marine intrusion. Winter conditions may significantly influence recruitment through altered reproductive timing, magnitude, and/or larval quality through parental carryover effects. We have expanded the introduction to discuss recruitment success/failures as possibly related to influences of winter conditions on larval production & viability.

As a side note, in Wasson et al. 2016 you and colleagues used multivariate analyses to assess environmental conditions and how they impacted larval recruitment patterns across years & sites. According to the supplementary materials, the environmental data used was mean monthly values averaged across “January to September for each oyster recruitment sampling year.” It would be interesting to do the same analysis with data from 1) winter months preceding recruitment sampling years, and 2) fall + winter months preceding. Perhaps fall &/or winter months influenced spring recruitment through direct changes to adult reproductive capacity or timing, or indirect through parental carryover effects.

---

**COMMENT:** Another application to potentially reference is the field’s recent focus on increasing intraspecific diversity of organisms that are being restored so that effects of “diversity” or identity can potentially emerge given in unpredicted environmental settings. I think this was briefly touched on at the end of the paper, but it would be nice to highlight it in the Introduction.

**RESPONSE:** Intraspecific diversity, and how we interpret results from studies using one vs. multiple “groups” of organisms (e.g. from one location) was definitely a consideration in our study. As such, we included oysters from 3 populations that are known to differ physiologically to capture responses across multiple phenotypes. To highlight the advantage of using multiple populations, I moved related content to its own paragraph in the introduction, and expanded a bit. In addition, I included the following recommendation, based on our evidence of positive carryover, in the conclusion: “Furthermore, conserving and restoring *O. lurida* in a variety of settings – including hypoxic, warmer, and less alkaline areas– could increase the probability that future populations are equipped for challenging conditions through selection or intergenerational carryover.”

Papers describing intraspecific variation among the Puget Sound Olympia populations that we examined:

- Heare, J. E., Blake, B., Davis, J. P., Vadopalas, B., & Roberts, S. B. (2017). Evidence of *Ostrea lurida* Carpenter, 1864, population structure in Puget Sound, WA, USA. *Marine Ecology*, 38(5). <https://doi.org/10.1111/maec.12458>
- Heare, J. E., White, S. J., Vadopalas, B., & Roberts, S. B. (2018). Differential response to stress in *Ostrea lurida* as measured by gene expression. *PeerJ*, 6, e4261. <https://doi.org/10.7717/peerj.4261>
- Silliman, K. E., Bowyer, T. K., & Roberts, S. B. (2018). Consistent differences in fitness traits across multiple generations of Olympia oysters. *Scientific Reports*, 8(1), 6080. <https://doi.org/10.1038/s41598-018-24455>

---

**COMMENT:** (1) For figure 6, please use symbols (in addition to gray-scale colors) to distinguish the different treatments

**RESPONSE:** Thank you for this suggestion, the plots were changed to include symbols to distinguish treatments. After testing grayscale, we decided to retain the colors, as they correspond to colors used throughout the manuscript, which we hope will help readers keep track of treatments. For instance, the experimental timeline (Figure 2) shows that only blue colors (6°C treatments) were tested during the field deployment.

---

**COMMENT:** (2) Please explain why only the pCO<sub>2</sub> factor was included in the outplant studies? I understand the logistics of this were probably quite difficult, but the lack of mention of why this one factor was dropped from the outplant study seemed curious.

**RESPONSE:** Unfortunately we did not have enough capacity and resources to test all 4 treatments and 4 cohorts across 4 bays with sufficient replication. We therefore opted to focus on one parental factor (pCO<sub>2</sub>) across multiple populations and locations.

To clarify for the reader, the methods section now includes the following statement: "To focus on the effect of parental pCO<sub>2</sub>, only offspring from 6°C parents were tested in the field."

---

**COMMENT:** (3) In line 367, I believe "among" should be inserted between "survival" and "bays"

**RESPONSE:** Thank you, "among" has been inserted as suggested.

---

**COMMENT:** (4) I may have missed this, but model-selection approach could be used to evaluate which of the environmental factors best explain the spatial variation in oyster survival from the field

**RESPONSE:** I performed this analysis as suggested. Using model selection, mean temperature, mean pH, and DO standard deviation were retained and were significant factors predicting survival. While this is interesting, I hesitate to draw conclusions in this manuscript about which conditions are optimal for juvenile Olympia oysters without more sites and environmental data. I have included boxplots of juvenile survival ~ environmental metric in the supplementary, and the associated GitHub repository contains data and R code so other researchers can access.

---

1    **Running Head: *Carryover effects in the Olympia oyster***

2    **Title: *Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations***

3    Laura H Spencer<sup>1</sup>, Yaamini R Venkataraman<sup>1</sup>, Ryan Crim<sup>2</sup>, Stuart Ryan<sup>2</sup>, Micah J Horwith<sup>3</sup>,

4    Steven B Roberts<sup>1</sup>

5    **Corresponding author:** Steven B Roberts, [sr320@uw.edu](mailto:sr320@uw.edu), [206-685-3742](tel:206-685-3742)

---

<sup>1</sup>University of Washington, School of Aquatic and Fishery Sciences, 1122 NE Boat St, Seattle, WA 98105, United States

<sup>2</sup>Puget Sound Restoration Fund, 8001 NE Day Rd W, Bainbridge Island, WA 98110, United States

<sup>3</sup>Washington State Department of Natural Resources, 1111 Washington St SE, MS 47027, Olympia, WA 98504, United States

## 6 Abstract

7 Predicting how populations will respond to ocean change across generations is critical to  
8 effective conservation of marine species. One emerging factor is the influence of parental  
9 exposures on offspring phenotype, known as intergenerational carryover effects. Parental  
10 exposure may deliver beneficial or detrimental characteristics to offspring that can influence  
11 larval recruitment patterns, thus shaping how populations and community structure respond to  
12 ocean change. Impacts of adult exposure to elevated winter temperature and pCO<sub>2</sub> on  
13 reproduction and offspring viability were examined in the Olympia oyster (*Ostrea lurida*) using  
14 three populations of adult, hatchery-reared *O. lurida*, plus an additional cohort spawned from one  
15 of the populations. Oysters were sequentially exposed to elevated temperature (+4°C, at 10°C),  
16 followed by elevated pCO<sub>2</sub> (+2204 µatm, at 3045 µatm) during winter months. Male gametes  
17 were more developed after elevated temperature exposure and less developed after high pCO<sub>2</sub>  
18 exposure, but there was no impact on female gametes or sex ratios. Oysters previously exposed  
19 to elevated winter temperature released larvae earlier, regardless of pCO<sub>2</sub> exposure. Those  
20 exposed to elevated winter temperature as a sole treatment released more larvae on a daily basis,  
21 but when also exposed to high pCO<sub>2</sub> there was no effect. These combined results indicate that  
22 elevated winter temperature accelerates *O. lurida* spermatogenesis, resulting in earlier larval  
23 release and increased production, with elevated pCO<sub>2</sub> exposure negating effects of elevated  
24 temperature. Altered recruitment patterns may therefore follow warmer winters due to  
25 precocious spawning, but these effects may be masked by coincidental high pCO<sub>2</sub>. Offspring  
26 were reared in common conditions for one year, then deployed for three months in four estuarine  
27 bays with distinct environmental conditions. Offspring of parents exposed to elevated pCO<sub>2</sub> had

28 higher survival rates in two of the four bays. This carryover effect demonstrates that parental  
29 conditions can have substantial ecologically relevant impacts that should be considered when  
30 predicting impacts of environmental change. Furthermore, Olympia oysters may be more  
31 resilient in certain environments when progenitors are pre-conditioned in stressful conditions.  
32 Combined with other recent studies, our work suggests that the Olympia may be more equipped  
33 than other oysters for the challenge of a changing ocean.

34 **Keywords:** *Ostrea lurida*, acidification, pH, warming, winter, reproduction, phenology,  
35 intergenerational, transgenerational, climate change

## 36 Introduction

37 The repercussions of ocean warming and acidification on marine invertebrate physiology  
38 are complex, but significant recent advances indicate that larval stages of marine taxa are  
39 particularly vulnerable (Byrne & Przeslawski, 2013; Kurihara, 2008; Przeslawski, Byrne, &  
40 Mellin, 2015). Understanding how shifting conditions will influence larval recruitment patterns  
41 is critical to predicting changing population dynamics, and thus community structure. One  
42 emerging consideration is whether larval stages benefit from ancestral exposures, based on  
43 evidence that memory of environmental stressors can be transferred between generations through  
44 non-genetic inheritance (reviewed in Perez & Lehner, 2019; Donelson *et al.* 2018; Eirin-Lopez  
45 & Putnam, 2019; Ross, Parker, & Byrne, 2016). Beneficial, or positive, carryover effects may be  
46 important acclimatory mechanisms for marine organisms facing rapid change, particularly those  
47 that evolved in dynamic environments like estuaries and the intertidal (Donelson, Salinas,  
48 Munday, & Shama, 2018; Gavery & Roberts, 2014). These carryover effects are defined as

49 transgenerational when they persist in generations that were never directly exposed.

50 Intergenerational, or parental, effects may be due to direct exposure as germ cells (Perez &  
51 Lehner, 2019). Trans- and intergenerational carryover effects are increasingly reported across  
52 marine phyla, including Cnidaria (e.g. Putnam & Gates, 2015), Echinodermata (e.g. Clark *et al.*,  
53 2019), Mollusca (e.g. Parker *et al.* 2015), Arthropoda (e.g. Thor & Dupont, 2015), and Chordata  
54 (Review: Munday 2014).

55 A foundational series of studies on the Sydney rock oyster (*Saccostrea glomerata*)  
56 provide strong evidence for intergenerational carryover effects in bivalves, an ecologically and  
57 economically important group of taxa (Dumbauld, Ruesink & Rumrill, 2009). Adult *S.*  
58 *glomerata* exposed to high pCO<sub>2</sub> produced larger larvae that were less sensitive to high pCO<sub>2</sub>,  
59 and the effect persisted in the successive generation (Parker *et al.*, 2012, 2015). In the presence  
60 of secondary stressors, however, parental high pCO<sub>2</sub> exposure rendered larvae more sensitive  
61 (Parker *et al.*, 2017). Intergenerational carryover effects are increasingly documented in larvae  
62 across other bivalve species, and are beneficial in the mussels *Mytilus chilensis* (Diaz *et al.*,  
63 2018) and *Mytilus edulis* (but not juveniles) (Kong *et al.*, 2019; Thomsen *et al.*, 2017), and  
64 detrimental in the clam *Mercenaria mercenaria*, the scallop *Argopecten irradians* (Griffith &  
65 Gobler, 2017), and the oyster *Crassostrea gigas* (Venkataraman, Spencer, & Roberts, 2019).

66 These preliminary studies provide strong evidence for intergenerational carryover effects  
67 in bivalves, but the body of work is still narrow in scope. Nearly all studies have exposed parents  
68 to stressors during denovo gamete formation (gametogenesis). For many temperate bivalve  
69 species, this occurs seasonally in the spring (Bayne, 1976). Yet, challenging periods of  
70 acidification and warming can occur during other times of the year (Evans, Hales, & Strutton,  
71 2013; Joesoef, Huang, Gao, & Cai, 2015; McGrath, McGovern, Gregory, & Cave, 2019). The

72 most corrosive carbonate environment in the Puget Sound estuary in Washington State, for  
73 example, commonly occurs in the winter when many species are reproductively inactive, while  
74 favorable conditions are in the spring when gametogenesis coincides with phytoplankton blooms  
75 (Pelletier, Roberts, Keyzers, & Alin, 2018). Thus, adult exposure to severely corrosive  
76 conditions during gametogenesis may not represent the natural estuarine system. To our  
77 knowledge, only one study has assessed carryover effects of exposure to acidification before  
78 reproductive conditioning in a bivalve, the oyster *C. gigas*, and found negative maternal  
79 carryover effects on larval survival (Venkataraman, Spencer, & Roberts, 2019), indicating that  
80 pre-gametogenic exposure also matters. No studies have yet attempted to examine  
81 intergenerational carryover effects of combined winter warming and acidification in bivalves.

82 To best predict whether intergenerational carryover effects will be beneficial or  
83 detrimental, it is also crucial to understand how warming and acidification will impact fertility  
84 and reproductive phenology. Temperature is a major driver of bivalve reproduction, and  
85 modulates gametogenesis (Joyce, Holthuis, Charrier, & Lindegarth, 2013; Maneiro, Pérez-  
86 Parallé, Pazos, Silva, & Sánchez, 2016; Oates, 2013), influences sex determination (Santerre *et*  
87 *al.*, 2013) and, in many species, triggers spawning (Fabioux, Huvet, Le Souchu, Le Pennec, &  
88 Pouvreau, 2005) (alongside other factors such as photoperiod, nutrition, lunar/tidal phases).  
89 Year-round warming may result in unexpected impacts to larval competency resulting from  
90 changes to reproduction. For instance, some temperate bivalve species have a thermal threshold  
91 for gametogenesis and enter a period of reproductive inactivity, or “quiescence”, which is  
92 believed necessary for successive spawning (Giese, 1959; Hopkins, 1937; Loosanoff, 1942).  
93 Warmer winters brought on by global climate change (IPCC, 2013, 2019) may therefore shift  
94 species’ reproductive cycles to begin earlier, or eliminate seasonality altogether, resulting in

95 poorly provisioned or ill-timed larvae (Chevillot *et al.*, 2017). Such impacts were clearly  
96 demonstrated using a long-term dataset (1973-2001) of estuarine clam *Macoma balthica*  
97 reproduction and temperature. Mild winters and earlier springs resulted in low fecundity, earlier  
98 spawning, and poor recruitment, which was largely explained by a phenological mismatch  
99 between spawning and peak phytoplankton blooms (Philippart *et al.*, 2003). The impacts of  
100 winter acidification on estuarine bivalve reproduction are less predictable. The few studies to  
101 date show that high pCO<sub>2</sub> delays gametogenesis in the oysters *Crassostrea virginica* and *S.*  
102 *glomerata* (Boulais *et al.*, 2017; Parker *et al.*, 2018), but both studies exposed oysters during  
103 gametogenesis. Acidification during the winter months could increase energetic requirements  
104 (Sokolova, Frederich, Bagwe, Lannig, & Sukhotin, 2012), and deplete glycogen reserves that are  
105 later utilized for gametogenesis in the spring (Mathieu & Lubet, 1993), but this hypothesis has  
106 yet to be tested.

107 The purpose of this study was to assess whether warmer, less alkaline winters will affect  
108 fecundity and offspring viability in the Olympia oyster, *Ostrea lurida*. The Olympia is native to  
109 the Pacific coast of North America (McGraw, 2009). Overharvest and pollution devastated  
110 populations in the early 1900s, and today 2-5% of historic beds remain (Blake & Bradbury,  
111 2012; Polson & Zacherl, 2009). Restoration efforts are afoot, but *O. lurida* populations continue  
112 to struggle, and may be further challenged by changing conditions (Barton, Hales, Waldbusser,  
113 Langdon, & Feely, 2012; Feely, Klinger, Newton, & Chadsey, 2012; Feely, Sabine, Hernandez-  
114 Ayon, Ianson, & Hales, 2008). For instance, large interannual variability in larval recruitment  
115 and frequent recruitment failures were recently reported (Wasson *et al.*, 2016; Kimbro, White &  
116 Grosholz, 2019). This variability is presumably related to inconsistent spawning success, larval  
117 survival, and retention, and governed predominantly by local conditions (Kimbrow, White &

118 Grosholz, 2019). It is unknown how the intensity, timing, and duration of local environmental  
119 conditions can predict recruitment failure (Wasson *et al.*, 2016). If winter conditions  
120 significantly influence recruitment through direct changes to adult reproductive capacity or  
121 timing, or indirect changes through parental carryover effects, population densities and  
122 distributions will inevitably shift with conditions.

123 Another consideration in this study was the genetic composition of test organisms. *Ostrea*  
124 *lurida* exhibits varying phenotypes among distinct populations (Silliman, 2019), which can  
125 influence their sensitivity to environmental stressors (Bible & Sanford, 2016; Bible, Evans &  
126 Sanford, 2019). Indeed, the two groups to measure the response of *O. lurida* larvae to ocean  
127 acidification found contrasting results – no effect (Waldbusser *et al.*, 2016), and slower growth  
128 (Hettinger *et al.*, 2012, 2013) – possibly a result of local adaptation. The source population used  
129 for experimental studies may therefore be a critical factor influencing climate-related findings.  
130 Furthermore, testing genetically diverse organisms could reveal cryptic genetic variation, alleles  
131 that confer stress resilience only under certain settings (Paaby & Rockman, 2014; Bitter *et al.*,  
132 *preprint*), which has implications for how wild populations are restored. Therefore, we tested  
133 three phenotypically distinct Puget Sound populations (Heare, Blake, Davis, Vadopalas, &  
134 Roberts, 2017; Silliman, Bowyer, & Roberts, 2018), which were hatchery-reared in common  
135 conditions to adulthood, to account for intraspecific variation while controlling for within-  
136 generation carryover effects (Hettinger *et al.*, 2012, 2013).

137 Our study is the first to assess the combined effects of elevated winter temperature and  
138 pCO<sub>2</sub> on reproduction, and to explore intergenerational carryover in an *Ostrea* spp. We exposed  
139 adult *O. lurida* to elevated temperature (+4°C), followed by elevated pCO<sub>2</sub> (+2204 µatm, -0.51  
140 pH). Gonad development, reproductive timing, and fecundity were assessed for the adults in the

141 laboratory, and offspring performance was assessed in the field. Elevated winter temperature was  
142 expected to impede gametogenic quiescence, presumably a critical annual event, subsequently  
143 reducing larval production. This prediction was in part based on observations of low larval yields  
144 in an *O. lurida* restoration hatchery (Ryan Crim, *unpublished*) following the winter 2016 marine  
145 heat wave in the Northeast Pacific Ocean (Gentemann, Fewings, & García-Reyes, 2017).  
146 Similarly, we predicted that high pCO<sub>2</sub> exposure would result in negative impacts due to  
147 increased energy requirements for calcification and cellular maintenance. Finally, we predicted  
148 that negative impacts would be amplified upon exposure to both conditions. By assessing the  
149 effects of winter warming and acidification on reproduction and offspring viability in multiple  
150 Olympia oyster populations, we provide an ecologically relevant picture of how the species will  
151 respond to ocean change.

## 152 Methods

### 153 Adult oyster temperature and pCO<sub>2</sub> exposures

154 Four cohorts of adult *Ostrea lurida* were used in this study. Three of the cohorts were first-  
155 generation hatchery-produced (F1) oysters ( $32.1 \pm 5.0$  mm), all hatched in Puget Sound (Port  
156 Gamble Bay) in 2013 (Heare *et al.*, 2017). The broodstock used to produce these F1 oysters were  
157 wild, harvested from Fidalgo Bay in North Puget Sound (F), Dabob Bay in Hood Canal (D), and  
158 Oyster Bay in South Puget Sound (O-1) (O in Figure 1). These populations are considered  
159 phenotypically distinct subpopulations (Heare *et al.*, 2017; White, Vadopalas, Silliman, &  
160 Roberts, 2017). The fourth cohort (O-2,  $21.9 \pm 3.3$  mm) was second-generation, hatchery-  
161 produced in 2015 from the aforementioned Oyster Bay F1 cohort, from a single larval release  
162 pulse and thus likely one family (Silliman, Bowyer, & Roberts, 2018). The O-2 cohort was

163 included to examine whether reproductive and offspring traits were consistent across generations  
164 of a population, with the O-2 cohort being closely related to each other (siblings) and 2 years  
165 younger than the other cohorts. Prior to the experiment, all oysters were maintained in pearl nets  
166 in Clam Bay (C) for a minimum of 500 days.

### 167 **Temperature treatment**

168 Oysters were moved from Clam Bay (C) to the Kenneth K. Chew Center for Shellfish Research  
169 and Restoration for the temperature and pCO<sub>2</sub> experiments. Oysters were held in one of two  
170 temperature regimes ( $6.1 \pm 0.2^\circ\text{C}$  and  $10.2 \pm 0.5^\circ\text{C}$ ) for 60 days beginning December 6, 2016  
171 (Figure 2). The temperatures correspond to historic local winter temperature ( $6^\circ\text{C}$ ) in Clam Bay,  
172 and anomalously warm winter temperature ( $10^\circ\text{C}$ ) as experienced during 2014-2016 (Gentemann  
173 *et al.*, 2017). For the temperature exposure, oysters from each cohort (100 for O-1 and F cohorts,  
174 60 for D, and 300 for O-2) were divided into four bags, two bags per temperature, in two flow-  
175 through experimental tanks (50L - 1.2-L/min). Temperature in the  $6^\circ\text{C}$  treatment was maintained  
176 using an aquarium chiller, and unchilled water was used for the  $10^\circ\text{C}$  treatment. Temperatures  
177 were recorded continuously with water temperature data loggers.

### 178 **High pCO<sub>2</sub> treatment**

179 A differential pCO<sub>2</sub> exposure was carried out after the temperature treatment ended. Following a  
180 10-day gradual temperature increase for the  $6^\circ\text{C}$  treatment to  $10^\circ\text{C}$ , oysters were further divided  
181 and held at ambient pCO<sub>2</sub> ( $841 \pm 85 \mu\text{atm}$ , pH  $7.82 \pm 0.02$ ) or high pCO<sub>2</sub> ( $3045 \pm 488 \mu\text{atm}$ , pH  $7.31 \pm 0.02$ )  
182 for 52 days (February 16 to April 8, 2017, Figure 2). Animals were housed in six flow-  
183 through tanks (50-L - 1.2-L/min), with three replicate tanks per pCO<sub>2</sub> treatment and oyster

184 cohort. High pCO<sub>2</sub> treated water was prepared using CO<sub>2</sub> injection. Filtered seawater (1 μm) first  
185 recirculated through a reservoir (1,610-L) with a degassing column to equilibrate with the  
186 atmosphere, then flowed into treatment reservoirs (757-L) recirculating through venturi injectors.  
187 Durafet pH sensors and a Dual Input Analytical Analyzer monitored pH in treatment reservoirs  
188 with readings every 180 seconds. Using solenoid valves, CO<sub>2</sub> gas was injected through lines at  
189 15 psi in 0.4 second pulses if pH exceeded the 7.22 set point. Water pH was continuously  
190 monitored in experimental tanks using Durafet pH sensors, and temperature (10.4 ± 0.4°C) was  
191 measured using water temperature data loggers. Twice weekly, water samples (1-L) were  
192 collected from experimental tanks, and temperature (°C), salinity (PSU), and pH (mV, converted  
193 to pH<sub>T</sub>) were measured immediately using a digital thermometer, conductivity meter, and pH  
194 electrode, respectively. Simultaneously, discrete water samples (120-mL) were collected in  
195 duplicate from experimental tanks and preserved with HgCl (50-μL) for later total alkalinity  
196 measurements using a titrator. Standard pH curves were generated on each sampling day prior to  
197 pH measurements using TRIS buffer prepared in-house at five temperatures (Appendix S1:  
198 Section S1). Using the seacarb library in R, pCO<sub>2</sub>, dissolved organic carbon (DIC), calcite  
199 saturation ( $\Omega_{\text{calcite}}$ ), and aragonite saturation ( $\Omega_{\text{aragonite}}$ ) were calculated for days 5, 33, and 48  
200 (Appendix S1: Table S1).

201 During both temperature and pCO<sub>2</sub> treatments, all oysters were fed from a shared algae  
202 header tank daily with Shellfish Diet 1800® (300-500-mL, Reed Mariculture) diluted in ambient  
203 pCO<sub>2</sub> seawater (200-L, Helm & Bourne, 2004), dosed continuously with metering pumps.  
204 Experimental, reservoir, and algae tanks were drained and cleaned, and oysters were monitored  
205 for mortality and rotated within the experimental system twice weekly.  
206

207 **Adult reproductive development**

208 A subset of oysters was sampled for gamete stage and dominant sex immediately before and  
209 after pCO<sub>2</sub> treatments (Figure 2) to capture developmental differences among treatments. Puget  
210 Sound *O. lurida* reportedly enter reproductive quiescence and resorb residual gametes when  
211 temperatures are below 12.5°C (Hopkins 1936, 1937), however recent evidence of low-  
212 temperature brooding in Puget Sound (10.5°C, Barber *et al.* 2016) suggests that reproductive  
213 activity may occur during warm winters. Therefore, gonad tissue was sampled to estimate the  
214 following: 1) whether residual gametes were resorbed or developed during winter treatments; 2)  
215 whether temperature and pCO<sub>2</sub> influenced winter activity; 3) if male and female gametes  
216 responded similarly; and 4) if gonad responses correspond with fecundity. Prior to pCO<sub>2</sub>  
217 exposure, 15 oysters were sampled from O-1, O-2, and F cohorts, and 9 from the D cohort. After  
218 pCO<sub>2</sub> exposure, 9, 6, and 15 oysters were sampled from each treatment for O-1/F, D, and O-2  
219 cohorts, respectively (distributed equally among replicates tanks). Whole visceral mass was  
220 excised and preserved in histology cassettes using the PAXgene Tissue FIX System, then  
221 processed for gonad analysis by Diagnostic Pathology Medical Group, Inc. (Sacramento, CA).

222 Adult gonad samples were assigned sex and stage using designations adapted from (da  
223 Silva, Fuentes, & Villalba, 2009) (Appendix S1: Tables S2 & S3). Sex was assigned as  
224 indeterminate (I), male (M), hermaphroditic primarily-male (HPM), hermaphroditic (H),  
225 hermaphroditic primarily-female (HPF), and female (F). Gonad sex was collapsed into simplified  
226 male and female designations for statistical analyses (hermaphroditic-primarily male = male,  
227 hermaphroditic-primarily female = female). For stage assignment, male and female gametes  
228 were assigned separately due to the high frequency of hermaphroditism (50.8%). Dominant  
229 gonad stage was then assigned based on the sex assignment. The da Silva gonad stages were

230 applied for early gametogenesis (stage 1), advanced (stage 2), and ripe (stage 3). Departures  
231 from da Silva's stage 0, stage 4 (partially spawned), and stage 5 (fully spawned/resorbing) were  
232 as follows: stage 0 in this study represents empty follicles, or no presence of male or female  
233 gonad tissue; stage 4 represents both spawned and resorbing gonad; this method did not include a  
234 separate stage 5, due to the very high frequency of residual gametes, and no distinct partially  
235 spawned oysters (for gonad images see Appendix S1: Fig. S2 and Spencer *et al.* 2019).

236 Treatment effects on gonad tissue were assessed for all cohorts combined in 4 gonad  
237 metrics: 1) gonad stage of dominant sex, 2) male gonad tissue when present, 3) female gonad  
238 tissue when present, and 4) gonad sex-collapsed (Chi-square test of independence). To assess the  
239 effects of elevated winter temperature alone, gonad metrics were compared between 6°C and  
240 10°C treatments prior to pCO<sub>2</sub> treatment. To determine the effect of pCO<sub>2</sub> exposure, gonad  
241 metrics were compared between ambient and high pCO<sub>2</sub> after 52 days in pCO<sub>2</sub> treatments,  
242 including temperature interaction effects. To estimate whether gonad changed during pCO<sub>2</sub>  
243 treatment, metrics were compared before and after ambient and high pCO<sub>2</sub> treatments, including  
244 temperature interaction effects. P-values were estimated using Monte-Carlo simulations with  
245 1,000 permutations, and corrected using the Benjamini & Hochberg method and  $\alpha=0.05$   
246 (Benjamini & Hochberg, 1995).

247

## 248 **Larval production**

249 Following pCO<sub>2</sub> exposure, adult oysters were spawned to assess impacts of winter treatment on  
250 larval production timing and magnitude. Beginning on April 11, 2017 (Figure 2), oysters were  
251 reproductively conditioned by raising temperatures gradually (~1°C/day) to  $18.1 \pm 0.1^\circ\text{C}$  and fed  
252 live algae cocktail at  $66,000 \pm 12,000$  cells/mL. Oysters spawned in the hatchery for 90 days

253 volitionally, i.e. naturally releasing gametes without chemical or physical manipulation. Six  
254 spawning tanks were used for each temperature x pCO<sub>2</sub> treatment: 6°C-high pCO<sub>2</sub>, 6°C-ambient  
255 pCO<sub>2</sub>, 10°C-high pCO<sub>2</sub>, and 10°C-ambient pCO<sub>2</sub>. Within the six tanks per treatment, two  
256 spawning tanks contained the F cohort (14-17 oysters), two tanks the O-1 cohort (14-17 oysters),  
257 one tank the D cohort (9-16 oysters), and one tank the O-2 cohort (111-126 oysters). More O-2  
258 oysters were used due to their small size. Olympia oysters release sperm, but have internal  
259 fertilization and release veliger larvae following a ~2 week brooding period (Coe ,1931;  
260 Hopkins, 1937). Therefore, production was assessed by collecting veliger larvae upon maternal  
261 release. Spawning tank outflow was collected in 7.5-L buckets using 100 µm screens made from  
262 15.25 cm polyvinyl chloride rings and 100 µm nylon mesh.

263 Larval collection was assessed for differences in spawn timing and fecundity. Larvae,  
264 first observed on May 11, 2017 (Figure 2), were collected from each spawning tank every one or  
265 two days for 60 days. The number of larvae released was estimated by counting and averaging  
266 triplicate subsamples of larvae homogenized in seawater. The following summary statistics were  
267 compared between temperature x pCO<sub>2</sub> treatments: total larvae released across the 90-day  
268 period, average number of larvae collected on a daily basis (excluding days where no larvae were  
269 released), maximum larvae released in one day, date of first release, date of maximum release,  
270 and number of substantial release days (greater than 10,000 larvae). The total and daily release  
271 values were normalized by the number of broodstock \* average broodstock height (cm), which  
272 can impact fecundity. Distributions were assessed using `qqp` in the `car` package for R (Fox &  
273 Weisberg, 2011), and log-transformed to meet normal distribution assumptions, if necessary.  
274 Differences between treatments were assessed using linear regression and Three-Way ANOVA  
275 (cohort was included as a covariate) with backwards deletion to determine the most

276 parsimonious models. Tukey Honest Significant Differences were obtained using TukeyHSD to  
277 assess pairwise comparisons (R Core Team, 2016). Dates of peak larval release were also  
278 estimated for each pCO<sub>2</sub> x temperature treatment by smoothing using locally weighted  
279 regression, with `geom_smooth` in the `ggplot` package (Wickham, 2017), with `span=0.3` and  
280 `degree=1`.

281

## 282 Offspring survival in a natural setting

283 To assess potential carryover effects of parental pCO<sub>2</sub> exposure, offspring from parents in 6°C-  
284 ambient pCO<sub>2</sub> and 6°C-high pCO<sub>2</sub> treatments were reared then deployed in the natural  
285 environment. To focus on the effect of parental pCO<sub>2</sub> exposure, only offspring from 6°C parents  
286 were tested in the field (Figure 2). Larvae were collected between May 19 and June 22, 2017,  
287 separated by parental pCO<sub>2</sub> exposure and cohort, and reared in common conditions for  
288 approximately 1 year (Figure 2; for rearing methods see Appendix S1: Section S6). On June 12,  
289 2018 the juveniles were placed in four bays in Puget Sound — Fidalgo Bay, Port Gamble Bay,  
290 Skokomish River Delta, and Case Inlet — with two sites per bay, for a total of eight locations  
291 (Figure 1). Autonomous sensors collected continuous water quality data at each location for pH,  
292 salinity (via conductivity), dissolved oxygen, temperature, and chlorophyll. For the F/D and O-  
293 1/O-2 cohorts, respectively, 30 and 10 oysters were placed at each location. Initial shell height  
294 and group weight were measured, then oysters were enclosed in mesh pouches and affixed inside  
295 shellfish bags to exclude predators. At the end of three months, survival, shell height and group  
296 weight were measured for live oysters.

297 Juvenile oyster survival was compared among bays and parental pCO<sub>2</sub> exposure with a  
298 binomial generalized linear mixed model (`glmm`) using `glmer` from the `lme4` package (vs. 1.1-

299 19). Chi-square tests compared survival differences among factors using the `car` package  
300 Anova function (Fox & Weisberg, 2011). Mean shell growth was determined by subtracting  
301 pre-deployment mean height from post-deployment mean height (not including dead oysters).  
302 Both mean shell growth and mass change were compared among factors using ANOVA and F-  
303 statistics to test differences by bay, parental pCO<sub>2</sub>, and cohort.

304

305 Make and model details for instruments used during treatments and field deployments are  
306 available in the Appendix S1: Section S2. All data analysis was performed in R version 3.3.1  
307 using the RStudio interface (R Core Team, 2016). Code for statistical analyses can be found in  
308 the associated Github repository (Spencer *et al.*, 2019).

309

## 310 **Results**

### 311 **Adult reproductive development**

312 After 60 days in temperature treatments ( $6.1 \pm 0.2^\circ\text{C}$  and  $10.2 \pm 0.5^\circ\text{C}$ ), gonad stage of the  
313 dominant sex differed significantly between temperatures (Table 2). The  $10^\circ\text{C}$  oysters had more  
314 instances of advanced gametogenesis (stage 2), and fewer that were resorbing/spawned (stage 4)  
315 (Figure 3). This difference was influenced strongly by more advanced male gametes in  $10^\circ\text{C}$   
316 oysters, but there were no differences in female gamete stages. No differences in sex ratio were  
317 observed between temperature treatments (Figure 4).

318 After 52 days in pCO<sub>2</sub> treatments, gonad stage of the dominant sex differed significantly  
319 between ambient and high pCO<sub>2</sub> in the oysters previously held in  $10^\circ\text{C}$  (Table 2). More mature  
320 gametes (stage 3) were found in  $10^\circ\text{C}$ -ambient pCO<sub>2</sub> (49%) compared to  $10^\circ\text{C}$ -high pCO<sub>2</sub>  
321 (33%). This difference was strongly influenced by oysters that were predominantly male, as male

322 gamete stage tended to differ between pCO<sub>2</sub> treatment, but female gamete stage did not (Table 2,  
323 Figure 3). In 6°C-treated oysters, there were no pCO<sub>2</sub> effects on gonad stage of the dominant sex,  
324 male gamete stage, or female gamete stage. No gonad stage or sex ratio differences were  
325 detected among oysters from 10°C-high pCO<sub>2</sub> (combined stressors) and 6°C-ambient pCO<sub>2</sub> (no  
326 stressors). Gonad sex did not differ significantly among treatments, however oysters tended to  
327 contain fewer male-only and more female-only gonad tissues in the riper, ambient pCO<sub>2</sub>-treated  
328 groups than male-only tissues (Figure 4).

329 Compared to oysters before pCO<sub>2</sub> exposure, those exposed to high pCO<sub>2</sub> did not differ in  
330 gonad sex, stage of the dominant sex, or female gamete stage. Male gametes in the 6°C treated  
331 oysters developed while in the high pCO<sub>2</sub> exposure, but there was no change in the 10°C treated  
332 oysters. Oysters held in ambient pCO<sub>2</sub> had significantly more advanced gonad compared to  
333 before CO<sub>2</sub> exposure regardless of temperature, again influenced strongly by changes in male  
334 gamete stage (Table 2).

335 No sampled oysters contained brooded embryos or larvae. Gonad data and patterns  
336 within cohorts is reported in Appendix S1: Figures S3, S4, and Table S4.

337

### 338 **Larval production**

339 Adults exposed to 10°C produced more larvae on a daily basis (excluding days where no larvae  
340 were released) than those exposed to 6°C in ambient pCO<sub>2</sub>-exposed oysters ( $p=0.040$ ), but not in  
341 high pCO<sub>2</sub>-exposed oysters ( $p=0.66$ ) (Figure 6, pCO<sub>2</sub>:temperature interaction: ( $F(2,8)=5.1$ ,  
342  $p=0.037$ ). Total larvae released over the 90-day spawning period tended to differ by treatment,  
343 but not significantly (temperature:pCO<sub>2</sub> interaction ( $F(2,8)=4.0$ ,  $p=0.063$ )). Temperature and  
344 pCO<sub>2</sub> as single factors did not affect total larvae released or daily averages.

345           The date of first larval release differed by temperature regardless of pCO<sub>2</sub> (Figures 5 & 6,  
346 F(1,8)=11.9, p=0.0087), and pCO<sub>2</sub> had no effect on timing (not retained in model). Onset was on  
347 average 5.2 days earlier in the 10°C treatment. Timing of peak larval release also differed by  
348 temperature treatment regardless of pCO<sub>2</sub> (Figure 6, F(3,19)=6.7, p=0.018), occurring on average  
349 8.3 days earlier in 10°C oysters. The 10°C treated oysters produced more large pulses of larvae,  
350 on average 2 additional days, than 6°C (F(1,8)=7.25, p=0.027).

351           In total, 18.5 million larvae were collected from 767 oysters. Total larvae produced by  
352 each treatment was 3.1M, 4.8M, 5.9M, and 4.5M for 6°C-ambient pCO<sub>2</sub>, 6°C-high pCO<sub>2</sub>, 10°C-  
353 ambient pCO<sub>2</sub>, and 10°C-high pCO<sub>2</sub>, respectively. Based on reports of approximately 215,000  
354 larvae produced per adult *O. lurida* of shell height 35 mm (Hopkins, 1936), the number of  
355 oysters that spawned as female in this study was approximately 86, with 14.3, 22.5, 27.6, and  
356 21.0 from the 6°C-ambient pCO<sub>2</sub>, 6°C-high pCO<sub>2</sub>, 10°C-ambient pCO<sub>2</sub>, and 10°C-high pCO<sub>2</sub>  
357 treatments, respectively. This estimate is likely low across all treatments, due to the smaller D  
358 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  
359 mm, respectively), therefore the total number of oysters that spawned as female and released  
360 larvae is likely higher than 86.

361           Larval production and timing data, including differences among cohorts, are included in  
362 Appendix S1: Section S5 and Table S5.

363

#### 364           **Offspring survival in a natural setting**

365           Juvenile survival after three months in the field was on average 15% higher in cohorts from high  
366 pCO<sub>2</sub> exposed parents than from ambient pCO<sub>2</sub> parents (44±37%, and 29±27%, respectively,  
367  $\chi^2=10.6$ , p=0.0011). The influence of parental pCO<sub>2</sub> on survival varied by bay (bay:parental

368 pCO<sub>2</sub> interaction  $\chi^2=15.3$ ,  $p=1.6e-3$ ), and by cohort (cohort:parental pCO<sub>2</sub> interaction  $\chi^2=23.5$ ,  
369  $p=3.2e-5$ ) (Table 3).

370 Survival in offspring from high pCO<sub>2</sub> parents was higher in the Fidalgo Bay and Port  
371 Gamble Bay locations ( $\chi^2=17.7$ ,  $p=2.6e-5$ ;  $\chi^2=10.0$ ,  $p=1.6e-3$ , respectively), but this was not  
372 the case in Skokomish River Delta or Case Inlet. Survival in the F cohort was 38% higher in  
373 oyster from pCO<sub>2</sub> parents than those from ambient pCO<sub>2</sub> parents across all deployment bays  
374 ( $\chi^2=28.1$ ,  $p=4.6e-7$ ), and within the Fidalgo Bay location ( $\chi^2=17.6$ ,  $p\text{-adj}=0.0001$ ). Survival in  
375 the D and O-1 cohorts did not differ significantly between parental pCO<sub>2</sub> across all bays (D:  
376  $\chi^2=0.4$ ,  $p=1$ , O-1:  $\chi^2=2.5$ ,  $p=0.44$ ), or within individual bays. More O-2 juveniles with ambient  
377 pCO<sub>2</sub> parents survived across all bays ( $\chi^2=9.1$ ,  $p=0.010$ ), and within the Skokomish River Delta  
378 ( $\chi^2=8.9$ ,  $p=0.011$ ).

379 Without considering parental pCO<sub>2</sub>, more oysters survived in Port Gamble Bay (mean  
380  $49\pm36\%$ ) and Fidalgo Bay ( $39\pm36\%$ ) than in Case Inlet (mean  $29\pm29\%$ ,  $p=0.012$  &  $p=0.037$ ,  
381 respectively) (bay factor,  $\chi^2=18.5$ ,  $p=3.4e-4$ ). Survival at Skokomish River Delta did not differ  
382 significantly from other locations ( $32\pm27\%$ ). No interaction between cohort and bay was  
383 detected ( $\chi^2=9.8$ ,  $p=0.37$ ) (Figure 7, Table 3).

384 Shell length was not affected by bay, cohort or parental pCO<sub>2</sub>. The mass per oyster  
385 (compared to before deployment) differed by cohort ( $F(3,76)=15.9$ ,  $p=4.0e-8$ ), due to Dabob Bay  
386 cohort growing less than the other three cohorts ( $\Delta$  g/oyster: D=0.5, F=1.2, O-1=1.6, & O-  
387 2=1.0). Mass change also differed by bay ( $F(3,76)=4.8$ ,  $p=3.9e-3$ ) due to less growth in oysters  
388 placed at Fidalgo Bay than in Port Gamble Bay and Case Inlet ( $\Delta$  g/oyster: FB=0.7, PGB=1.0,  
389 CI=1.1, SK=0.8) (Appendix S1: Figure S5).

## 390 Discussion

391 Ocean acidification and ocean warming potentially threaten marine organisms, particularly  
392 ectothermic calcifiers (Hoffman *et al.* 2010). An organism's genotype, complete environmental  
393 history, and the timing and magnitude of environmental perturbations may all determine its  
394 fitness in future ocean conditions. To begin teasing apart these complex factors in the Olympia  
395 oyster, this study examined four adult cohorts with distinct genetic structure but known, shared  
396 histories. Elevated winter temperature resulted in increased gonad development, which  
397 corresponded with earlier and more frequent larval release (on average 5.2 days earlier, 2  
398 additional days). High pCO<sub>2</sub> exposure negatively influenced gonad maturation state, but did not  
399 affect subsequent fecundity. Offspring from parents exposed to elevated pCO<sub>2</sub> had higher overall  
400 survival upon deployment. Differences in juvenile survival among bays and cohorts indicate that  
401 carryover effects are dependent upon the environment and genotype, and reinforce the  
402 importance of using multiple sources of test organisms in stress-response studies.

## 403 Reproduction

404 We expected elevated winter temperature to reduce fecundity, based on predictions that changes  
405 to reproductive quiescence and metabolism would be deleterious to spring reproduction. Counter  
406 to this prediction, warm winter temperature positively affected larval production. Oysters in  
407 elevated temperature contained more developed male gametes after treatment, and subsequently  
408 began releasing larvae earlier and produced more larvae per day compared to cold-treated  
409 oysters. We find no evidence that cold winters are critical for spring reproduction, but rather  
410 elevated winter temperature may elongate the *O. lurida* spawning season. In comparison, a 29-  
411 year dataset of *M. balthica* reproduction showed that as winter temperature increased, spring  
412 spawning began earlier and fecundity declined (Philippart *et al.*, 2003). However, the present

413 study was conducted in a hatchery setting, with ample phytoplankton, and did result in a  
414 temperature shift during spawning. In the wild numerous additional abiotic and biotic factors will  
415 contribute to *O. lurida* fitness, and warmer winters may result in earlier and longer reproductive  
416 seasons only if nutritional requirements are met. Whether larvae released earlier in the spring can  
417 survive to recruitment will greatly depend on many factors including food availability and  
418 predation. Those modeling larval recruitment (e.g. Kimbro, White & Grosholz, 2019; Wasson *et*  
419 *al.*, 2016) should consider including winter temperature as a factor influencing spatiotemporal  
420 recruitment patterns.

421 We predicted that high pCO<sub>2</sub> exposure would redirect energy away from storage to  
422 maintenance processes, resulting in delayed gametogenesis and poor fecundity in the spring.  
423 After exposure to 3045 µatm pCO<sub>2</sub> (pH 7.31), fewer oysters contained ripe or advanced male  
424 gonad tissue than in ambient pCO<sub>2</sub>, signaling reduced spermatogenic activity. Female gonad, sex  
425 ratios, and subsequent fecundity were not affected by sole exposure to high pCO<sub>2</sub>. Similar  
426 impacts on gametogenesis during exposure were observed in the Sydney rock (*S. glomerata*) and  
427 Eastern (*C. virginica*) oysters, but with varying pCO<sub>2</sub> thresholds. Parker *et al.* (2018) found *S.*  
428 *glomerata* gametogenesis to slow in 856 µatm (pH 7.91), and Boulais *et al.* (2017) found normal  
429 rates at 2260 µatm (pH 7.5), delay at 5584 µatm (pH 7.1), and full inhibition at 18480 µatm (pH  
430 6.9) in *C. virginica*. Together, these studies indicate that high pCO<sub>2</sub> slows the rate of  
431 gametogenesis, but the level at which pCO<sub>2</sub> affects gametogenesis appears species-specific, and  
432 likely reflective of variable physiological mechanisms and reproductive strategies.

433 The combined effects of sequential elevated temperature and pCO<sub>2</sub> treatments did not act  
434 synergistically to delay gonad development, but instead resulted in oysters with gonad stage and  
435 fecundity no different from the untreated oysters. Similarly, combined simultaneous temperature

436 and high pCO<sub>2</sub> exposures did not affect *S. glomerata* fecundity (Parker *et al.*, 2018). We did  
437 detect a pCO<sub>2</sub> dependent effect of temperature on the average number of larvae released per day.  
438 Oysters that had previously been exposed to 10°C produced more larvae than 6°C, but only after  
439 ambient pCO<sub>2</sub> exposure, which may reflect a general reproductive arrest that occurs when  
440 exposed to high pCO<sub>2</sub>. Despite experimental differences (*e.g.* sequential vs. simultaneous  
441 exposures) which can influence outcomes (Bible *et al.* 2017), both Parker *et al.* (2018) and the  
442 present study indicate that high pCO<sub>2</sub> slows gametogenesis, elevated temperature accelerates it,  
443 and these two environmental drivers act antagonistically on gonad development if occurring in  
444 the same reproductive season. An important factor not included in either study is ecologically  
445 relevant variability. Temperature and pCO<sub>2</sub> oscillations, driven by tides and diurnal  
446 photosynthesis, could offer daily refuge or expose oysters to dynamic changes, altering how  
447 combined stressors interact (Cheng *et al.* 2015).

448 In contrast to prior studies, temperature and pCO<sub>2</sub> did not impact *O. lurida* sex ratios,  
449 whereas in high pCO<sub>2</sub> *C. virginica* skewed male (Boulais *et al.*, 2017), and *S. glomerata* skewed  
450 female (Parker *et al.*, 2018). This observation may be explained by very low incidence of total  
451 reproductive inactivity in our *O. lurida* cohorts — only four out of the 108 oysters that were  
452 sampled prior to pCO<sub>2</sub> treatment contained empty follicles — and thus sex ratios may be  
453 different if pCO<sub>2</sub> exposure occurs earlier in life during initial sex differentiation. Furthermore,  
454 high pCO<sub>2</sub> exposure only occurred in winter, prior to spawning. If high pCO<sub>2</sub> persists during  
455 oocyte maturation and spawning, *O. lurida* fecundity may be reduced similar to *C. virginica* and  
456 *S. glomerata*. Future research should examine *O. lurida* sexual development during the initial  
457 switch from male to female, which can occur the first winter after settlement (Moore *et al.*,

458 2016), and across a range of pCO<sub>2</sub> to determine conditions in which gametogenesis and sex  
459 determination are affected.

460 **Offspring**

461 Abiotic parental stressors can be beneficial, neutral, or detrimental to offspring viability  
462 (Donelson *et al.*, 2018). We explored carryover effects of adult exposure to winter pCO<sub>2</sub> on  
463 offspring by testing survival in the field. Offspring with high pCO<sub>2</sub> parental histories performed  
464 better in two of four locations, Fidalgo Bay and Port Gamble Bay. Carryover effects of parental  
465 high pCO<sub>2</sub> exposure may therefore be neutral, or beneficial, to offspring depending on the  
466 environmental conditions. Port Gamble Bay and Fidalgo Bay are more influenced by oceanic  
467 waters, which could explain cooler observed temperatures. These locations are also typically less  
468 stratified than the Skokomish River Delta and Case Inlet. In Port Gamble Bay, where pCO<sub>2</sub>  
469 parental history most significantly correlated with offspring survival across cohorts, mean pH  
470 was considerably lower than the other deployment locations (-0.17 pH units), and mean salinity  
471 was higher (+3.8 PSU). Given the experimental design we are able to clearly demonstrate that  
472 manifestation of carryover effects in Olympia oysters is dependent on environmental conditions.  
473 Specifically, there is a greater likelihood of beneficial carryover effects when parents are  
474 exposed to stressful conditions. Overall, carryover effects of parental pCO<sub>2</sub> treatment were  
475 positive, however negative effects were observed in the O-2 cohort. This discrepancy could  
476 relate to unique O-2 juvenile characteristics, as they were bred from siblings, and were 3rd-  
477 generation hatchery produced. The complex interactions among parental exposure, bay, and  
478 cohort indicate that offspring viability is influenced by ancestral environment history,  
479 environmental conditions, and genotype.

480 Our results contrast with a similar study that exposed *C. gigas* oysters to high pCO<sub>2</sub>  
481 during the winter, and found fewer hatched larvae 18 hours post-fertilization from exposed  
482 females, with no discernable paternal effect (Venkataraman, Spencer & Roberts, 2019). Hatch  
483 rate was not directly measured in this study due to the *O. lurida* brooding behavior; however, no  
484 difference in daily and total larvae released suggest that hatch rate was unaffected by pCO<sub>2</sub>. The  
485 different responses seen in Venkataraman, Spencer & Roberts (2019) and the present study may  
486 reflect variability among species and spawning method. *C. gigas* gametes were collected  
487 artificially by stripping gonad, whereas *O. lurida* late-stage veliger larvae were collected upon  
488 release from the brood chamber. For instance, volitionally-spawned gamete quality and  
489 fertilization rates could vary between the natural versus artificial settings to influence larval  
490 viability. Larval brooding may also be a mechanism by which sensitive larvae are acclimatized  
491 to stressors, as the *O. lurida* brood chamber pH and dissolved oxygen can be significantly lower  
492 than the environment (Gray *et al.*, *in press*).

493 Beneficial parental carryover may also be linked to the male-specific gonad effects, and  
494 the conditions in which the adult oysters were held. During high pCO<sub>2</sub> exposure, oocyte stage  
495 and prevalence did not change, which indicates that oogenesis did not occur. Negative  
496 intergenerational carryover effects are commonly linked to variation in oocyte quality, which can  
497 be affected by the maternal environment during oogenesis (Utting & Millican, 1997). In the  
498 Chilean flat oyster (*Ostrea chilensis*), for instance, egg size and lipid content positively correlate  
499 with juvenile growth and survival (Wilson, Chaparro, & Thompson, 1996). If high pCO<sub>2</sub>  
500 exposure were to coincide with oocyte proliferation and growth, *O. lurida* egg quality and larval  
501 viability could be compromised. In contrast, male gonad stage advanced significantly during  
502 pCO<sub>2</sub> exposure. Intergenerational and transgenerational carryover effects are increasingly linked

503 to the paternal environment in other taxa, such as inheritance of epigenetic changes to the male  
504 germ line (Rodgers, Morgan, Bronson, Revello, & Bale, 2013; Anway, 2005; Soubry, Hoyo,  
505 Jirtle, & Murphy, 2014). Positive carryover effects of environmental stressors observed in this  
506 and other marine invertebrate taxa may be due to paternal epigenetic effects, but this link has not  
507 yet been observed.

508

## 509 Conclusion

510 This study clearly demonstrates that exposure to elevated winter temperature and altered  
511 carbonate chemistry impacts reproduction and offspring viability in the Olympia oyster.  
512 Furthermore, we report the first observations of intergenerational plasticity in an *Ostrea* species,  
513 that is dependent on offspring environmental conditions and population. The observed context-  
514 dependent carryover effects could have a substantial impact on species resilience. Combined  
515 with previous reports of resilience to environmental stressors (Waldbusser *et al* 2016; Cheng *et*  
516 *al.* 2017) and intraspecific variability (Bible, Evans & Sanford, 2019; Maynard, Bible, Pespeni,  
517 Sanford, & Evans, 2018; Silliman, Bowyer, & Roberts, 2018; Heare, Blake, Davis, Vadopalas, &  
518 Roberts, 2017), the Olympia oyster may be more capable than other marine bivalve species to  
519 withstand and adapt to unprecedented ocean change. Furthermore, conserving and restoring *O.*  
520 *lurida* in a variety of settings — including hypoxic, warmer, and less alkaline areas — could  
521 increase the probability that future populations are equipped for challenging conditions through  
522 selection or intergenerational carryover.

523 As temperatures rise and ocean acidification progresses, there may be profound and  
524 unexpected seasonal changes across marine taxa. Accurate predictions will need to consider  
525 parental carryover effects, as they can impart neutral, beneficial, or detrimental characteristics to

526 offspring, which depend on complex interactions among parental exposure timing, reproductive  
527 strategies, species plasticity, and standing genetic structure. With these considerations, future  
528 biological response studies need to be aware of three possible factors influencing results: 1)  
529 source population; 2) environmental history (within-lifetime carryover effects); and 3) ancestral  
530 environmental history (inter- and transgenerational carryover effects). Controlling for, or at  
531 minimum recognizing and recording these factors, will provide important context for those  
532 predicting ecosystem response to environmental change.

### 533 **Acknowledgements**

534 Our gratitude to the following people who assisted with this project: Grace Crandall, Kaitlyn  
535 Mitchell, Olivia Smith, Megan Hintz, Rhonda Elliott, Lindsay Alma, Duncan Greeley, Beyer and  
536 Jackson Roberts, and Ian Davidson helped with oyster husbandry and sampling; Alice Helker  
537 advised on husbandry and larval rearing system engineering; Emily Kunselman helped manage  
538 the field deployment; Sam White and Hollie Putnam contributed to the carbonate chemistry  
539 analysis; Katherine Silliman and Jake Heare produced (and saved) the experimental oysters; the  
540 NOAA Manchester Research Center and Puget Sound Restoration Fund provided facilities and  
541 materials; committee members Jackie Padilla-Gamiño and Rick Goetz advised and supported  
542 this extended project. Thank you to David Kimbro and an anonymous reviewer for constructive  
543 comments on the manuscript.

544 This work was supported in part by the National Science Foundation Graduate Research  
545 Fellowship Program, the National Shellfisheries Association Melbourne R. Carriker Student  
546 Research Grant, Washington State Department of Natural Resources, and a grant from

547 Washington Sea Grant, University of Washington, pursuant to the National Oceanic and  
548 Atmospheric Administration Award No. NA14OAR4170078; Project R/SFA-8. The views  
549 expressed herein are those of the author(s) and do not necessarily reflect the views of any  
550 funding agency.

551

## 552 **References**

- 553 Anway, M.D., Cupp, A. S., Uzumcu, M., & M. K. Skinner (2005). Epigenetic Transgenerational  
554 Actions of Endocrine Disruptors through the Male Germ-Line. *Science*, **308**(5727): 1466-  
555 1469. <https://doi.org/10.1126/science.1108190>
- 556 Barber, J. S., Dexter, J. E., Grossman, S. K., Greiner, C. M., & Mcardle, J. T. (2016). Low  
557 Temperature Brooding of Olympia Oysters (*Ostrea Lurida*) in Northern Puget Sound.  
558 *Journal of Shellfish Research* **35** (2): 351–57.
- 559 Barton, A., Hales, B., Waldbusser, G. G., Langdon, C., & Feely, R. A. (2012). The Pacific  
560 oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide  
561 levels: Implications for near-term ocean acidification effects. *Limnology and  
562 Oceanography*, **57**(3): 698–710. Retrieved from  
563 <https://onlinelibrary.wiley.com/doi/abs/10.4319/lo.2012.57.3.0698>
- 564 Bayne, B. L. (1976). Aspects of Reproduction in Bivalve Molluscs. In: M. Wiley (Ed.), Estuarine  
565 Processes (pp. 432–448). Academic Press. <https://doi.org/10.1016/B978-0-12-751801-5.50043-5>
- 567 Benjamini, Y., & Hochberg, Y. (1995). Controlling the False Discovery Rate: A Practical and  
568 Powerful Approach to Multiple Testing. *Journal of the Royal Statistical Society. Series B,  
569 Statistical Methodology*, **57**(1): 289–300. Retrieved from

- 570           <http://www.jstor.org/stable/2346101>
- 571   Bible, J. M., & Sanford, E. (2016). Local adaptation in an estuarine foundation species:  
572       Implications for restoration. *Biological Conservation*, **193**: 95–102.
- 573       <https://doi.org/10.1016/j.biocon.2015.11.015>
- 574   Bible, J. M., Evans, T. G., & Sanford, E. (2019). Differences in Induced Thermotolerance among  
575       Populations of Olympia Oysters. *Comparative Biochemistry and Physiology. Part A,*  
576       *Molecular & Integrative Physiology*, Online version published September, 110563.  
577       <https://doi.org/10.1016/j.cbpa.2019.110563>
- 578   Bitter, M. C., Kapsenberg, L., Gattuso, J. -P., & Pfister, C. A. (2019). Cryptic genetic variation  
579       underpins rapid adaptation to ocean acidification *BioRxiv* p. 700526.  
580       <https://doi.org/10.1101/700526>
- 581   Blake, B., & Bradbury, A. (2012). Washington Department of Fish and Wildlife plan for  
582       rebuilding Olympia oyster (*Ostrea lurida*) populations in Puget Sound with a historical and  
583       contemporary overview. Brinnon, WA: Washington Department of Fish and Wildlife.  
584       Retrieved from  
585       [https://www.westcoast.fisheries.noaa.gov/publications/aquaculture/olympia\\_oyster\\_restoration\\_plan\\_final.pdf](https://www.westcoast.fisheries.noaa.gov/publications/aquaculture/olympia_oyster_restoration_plan_final.pdf)
- 587   Boulais, M., Chenevert, K. J., Demey, A. T., Darrow, E. S., Robison, M. R., Roberts, J. P., &  
588       Volety, A. (2017). Oyster reproduction is compromised by acidification experienced  
589       seasonally in coastal regions. *Scientific Reports*, **7**(1), 13276.  
590       <https://doi.org/10.1038/s41598-017-13480-3>
- 591   Byrne, M., & Przeslawski, R. (2013). Multistressor impacts of warming and acidification of the  
592       ocean on marine invertebrates' life histories. *Integrative and Comparative Biology*, **53**(4):

- 593 582–596. <https://doi.org/10.1093/icb/ict049>
- 594 Chevillot, X., Drouineau, H., Lambert, P., Carassou, L., Sautour, B., & Lobry, J. (2017). Toward  
595 a phenological mismatch in estuarine pelagic food web? *PLoS One*, **12**(3): e0173752.  
596 <https://doi.org/10.1371/journal.pone.0173752>
- 597 Clark, M. S., Suckling, C. C., Cavallo, A., Mackenzie, C. L., Thorne, M. A. S., Davies, A. J., &  
598 Peck, L. S. (2019). Molecular Mechanisms Underpinning Transgenerational Plasticity in the  
599 Green Sea Urchin *Psammechinus miliaris*. *Scientific Reports*, **9**(1): 952.  
600 <https://doi.org/10.1038/s41598-018-37255-6>
- 601 Coe, W. R. (1931). Sexual Rhythm in the California Oyster (*Ostrea lurida*). *Science*, **74**(1914):  
602 247–249.
- 603 da Silva, P. M., Fuentes, J., & Villalba, A. (2009). Differences in gametogenic cycle among  
604 strains of the European flat oyster *Ostrea edulis* and relationship between gametogenesis  
605 and bonamiosis. *Aquaculture*, **287**(3–4): 253–265.  
606 <https://doi.org/10.1016/j.aquaculture.2008.10.055>
- 607 Diaz, R., Lardies, M. A., Tapia, F. J., Tarifeño, E., & Vargas, C. A. (2018). Transgenerational  
608 effects of pCO<sub>2</sub>-driven ocean acidification on adult mussels *Mytilus chilensis* modulate  
609 physiological response to multiple stressors in larvae. *Frontiers in Physiology*, **9**: 1349.  
610 <https://doi.org/10.3389/fphys.2018.01349>
- 611 Donelson, J. M., Salinas, S., Munday, P. L., & Shama, L. N. S. (2018). Transgenerational  
612 plasticity and climate change experiments: Where do we go from here? *Global Change  
613 Biology*, **24**(1): 13–34. <https://doi.org/10.1111/gcb.13903>
- 614 Dumbauld, B. R., Ruesink, J. L., & Rumrill, S. S. (2009). The ecological role of bivalve shellfish  
615 aquaculture in the estuarine environment: A review with application to oyster and clam

- 616 culture in West Coast (USA) estuaries. *Aquaculture*, **290**(3): 196–223.
- 617 Evans, W., Hales, B., & Strutton, P. G. (2013). pCO<sub>2</sub> distributions and air–water CO<sub>2</sub> fluxes in  
618 the Columbia River estuary. *Estuarine, Coastal and Shelf Science*, **117**: 260–272.  
619 <https://doi.org/10.1016/j.ecss.2012.12.003>
- 620 Fabioux, C., Huvet, A., Le Souchu, P., Le Pennec, M., & Pouvreau, S. (2005). Temperature and  
621 photoperiod drive *Crassostrea gigas* reproductive internal clock. *Aquaculture*, **250**(1–2):  
622 458–470. <https://doi.org/10.1016/j.aquaculture.2005.02.038>
- 623 Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., & Hales, B. (2008). Evidence for  
624 upwelling of corrosive “acidified” water onto the continental shelf. *Science*, **320**(5882):  
625 1490–1492. <https://doi.org/10.1126/science.1155676>
- 626 Feely, R. A., Klinger, T., Newton, J. A., & Chadsey, M. (2012). Scientific summary of ocean  
627 acidification in Washington State marine waters. NOAA OAR Special Report. Retrieved  
628 from <https://fortress.wa.gov/ecy/publications/documents/1201016.pdf>
- 629 Fox, J., & Weisberg, S. (2011). *An R Companion to Applied Regression*. SAGE Publications,  
630 Inc. Retrieved from <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>
- 631 Gavery, M. R., & Roberts, S. B. (2014). A context dependent role for DNA methylation in  
632 bivalves. *Briefings in Functional Genomics*, **13**(3): 217–222.  
633 <https://doi.org/10.1093/bfgp/elt054>
- 634 Gentemann, C. L., Fewings, M. R., & García-Reyes, M. (2017). Satellite sea surface  
635 temperatures along the West Coast of the United States during the 2014–2016 northeast  
636 Pacific marine heat wave: Coastal SSTs During “the Blob.” *Geophysical Research Letters*,  
637 **44**(1): 312–319. <https://doi.org/10.1002/2016GL071039>
- 638 Giese, A. C. (1959). Comparative physiology: annual reproductive cycles of marine

- 639 invertebrates. *Annual Review of Physiology*, **21**: 547–576.
- 640 <https://doi.org/10.1146/annurev.ph.21.030159.002555>
- 641 Gray, M. W., Chaparro O., Huebert K. B., O'Neill, S. P., Couture, T., Moreira A., Brady, D. C.
- 642 (*in press*). Does brooding prepare young for tomorrow's acidic oceans and estuaries?
- 643 *Journal of Shellfish Research*.
- 644 Griffith, A. W., & Gobler, C. J. (2017). Transgenerational exposure of North Atlantic bivalves to
- 645 ocean acidification renders offspring more vulnerable to low pH and additional stressors.
- 646 *Scientific Reports*, **7**(1): 11394. <https://doi.org/10.1038/s41598-017-11442-3>
- 647 Heare, J. E., Blake, B., Davis, J. P., Vadopalas, B., & Roberts, S. B. (2017). Evidence of *Ostrea*
- 648 *lurida* Carpenter, 1864, population structure in Puget Sound, WA, USA. *Marine Ecology*,
- 649 **38**(5): e12458. <https://doi.org/10.1111/maec.12458>
- 650 Heare, J. E., White, S. J., Vadopalas, B., & Roberts, S. B. (2018). Differential response to stress
- 651 in *Ostrea lurida* as measured by gene expression. *PeerJ*, **6**: e4261.
- 652 <https://doi.org/10.7717/peerj.4261>
- 653 Helm, M. M. & Bourne, N. (2004). Hatchery culture of bivalves: a practical manual. Food and
- 654 agriculture organization of the United Nations. Retrieved from <http://www.sidalc.net/cgi-bin/wxis.exe/?IsisScript=UACHBC.xis&method=post&formato=2&cantidad=1&expresion>
- 655 =mfn=102646
- 656
- 657 Hettinger, A., Sanford, E., Hill, T. M., Lenz, E. A., Russell, A. D., & Gaylord, B. (2013). Larval
- 658 carry-over effects from ocean acidification persist in the natural environment. *Global*
- 659 *Change Biology*, **19**(11): 3317–3326. Retrieved from <http://www.fao.org/3/a-y5720e.pdf>
- 660 Hettinger, A., Sanford, E., Hill, T. M., Russell, A. D., Sato, K. N., Hoey, J., Forsch, M., Page, H.
- 661 N., Gaylord, B. (2012). Persistent carry-over effects of planktonic exposure to ocean

- 662 acidification in the Olympia oyster. *Ecology*, **93**(12): 2758–2768.
- 663 <https://doi.org/10.1890/12-0567.1>
- 664 Hofmann, G. E., Barry, J. P., Edmunds, P. J., Gates, R. D., Hutchins, D. A., Klinger, T., &
- 665 Sewell, M. A. (2010). The Effect of Ocean Acidification on Calcifying Organisms in
- 666 Marine Ecosystems: An Organism-to-Ecosystem Perspective. *Annual Review of Ecology,*
- 667 *Evolution, and Systematics*, **41**: 127–147.
- 668 <https://doi.org/10.1146/annurev.ecolsys.110308.120227>
- 669 Hopkins, A. E. (1936). Ecological Observations on Spawning and Early Larval Development in
- 670 the Olympia Oyster (*Ostrea Lurida*). *Ecology*, **17**(4): 551–566.
- 671 <https://doi.org/10.2307/1932760>
- 672 Hopkins, A. E. (1937). Experimental observations on spawning, larval development, and setting
- 673 in the olympia oyster. *United States Bureau of Fisheries Bulletin*. **48**:438–503.
- 674 IPCC, 2013: Climate Change 2013: The Physical Science Basis. Contribution of Working Group
- 675 I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change
- 676 [Stocker, T.F., D. Qin, G.-K. Plattner, M. Tignor, S.K. Allen, J. Boschung, A. Nauels, Y.
- 677 Xia, V. Bex and P.M. Midgley (eds.)]. Cambridge University Press, Cambridge, United
- 678 Kingdom and New York, NY, USA, 1535 pp. doi:10.1017/CBO9781107415324.
- 679 IPCC, 2019: Summary for Policymakers. In: IPCC Special Report on the Ocean and Cryosphere
- 680 in a Changing Climate [H.-O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M.
- 681 Tignor, E. Poloczanska, K. Mintenbeck, M. Nicolai, A. Okem, J. Petzold, B. Rama, N.
- 682 Weyer (eds.)]. In press.
- 683 Joesoef, A., Huang, W.-J., Gao, Y., & Cai, W.-J. (2015). Air–water fluxes and sources of carbon
- 684 dioxide in the Delaware Estuary: spatial and seasonal variability. *Biogeosciences*, **12**(20):

- 685 6085–6101. <https://doi.org/10.5194/bg-12-6085-2015>
- 686 Joyce, A., Holthuis, T. D., Charrier, G., & Lindegarth, S. (2013). Experimental Effects of  
687 Temperature and Photoperiod on Synchrony of Gametogenesis and Sex Ratio in the  
688 European Oyster *Ostrea edulis* ( Linnaeus ). *Journal of Shellfish Research*, **32**(2): 447–458.  
689 <https://doi.org/10.2983/035.032.0225>
- 690 Kelly, M. W., Padilla-Gamiño, J. L., & Hofmann, G. E. (2013). Natural variation and the  
691 capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus*  
692 *purpuratus*. *Global Change Biology*, **19**(8): 2536–2546. <https://doi.org/10.1111/gcb.12251>
- 693 Kimbro, D. L., White, J. W., & Grosholz, E. D. (2019). The Dynamics of Open Populations:  
694 Integration of Top-down, Bottom-up and Supply-side Influences on Intertidal Oysters.  
695 *Oikos* **128**(4): 584–95, <https://doi.org/10.1111/oik.05892>
- 696 Kong, H., Jiang, X., Clements, J. C., Wang, T., Huang, X., Shang, Y., Chen, J., Hu, M., Wang,  
697 Y. (2019). Transgenerational effects of short-term exposure to acidification and hypoxia on  
698 early developmental traits of the mussel *Mytilus edulis*. *Marine Environmental Research*,  
699 **145**: 73–80. <https://doi.org/10.1016/j.marenvres.2019.02.011>
- 700 Kurihara, H. (2008). Effects of CO<sub>2</sub>-driven ocean acidification on the early developmental stages  
701 of invertebrates. *Marine Ecology Progress Series*, **373**: 275–284.  
702 <https://doi.org/10.3354/meps07802>
- 703 Loosanoff, V. L. (1942). Seasonal gonadal changes in the adult oysters, *Ostrea virginica*, of Long  
704 Island Sound. *The Biological Bulletin*, **82**(2): 195–206. <https://doi.org/10.2307/1538070>
- 705 Maneiro, V., Pérez-Parallé, M. L., Pazos, A. J., Silva, A., & Sánchez, J. L. (2016). Combined  
706 Effects of Temperature and Photoperiod on the Conditioning of the Flat Oyster (*Ostrea*  
707 *edulis* [Linnaeus, 1758]) in Winter. *Journal of Shellfish Research*, **35**(1): 137–141.

- 708        <https://doi.org/10.2983/035.035.0115>
- 709        Massamba-N'Siala, G., Prevedelli, D., & Simonini, R. (2014). Trans-generational plasticity in  
710        physiological thermal tolerance is modulated by maternal pre-reproductive environment in  
711        the polychaete *Ophryotrocha labronica*. *The Journal of Experimental Biology*, **217**(Pt 11):  
712        2004–2012. <https://doi.org/10.1242/jeb.094474>
- 713        Mathieu, M., & Lubet, P. (1993). Storage tissue metabolism and reproduction in marine  
714        bivalves—a brief review. *Invertebrate Reproduction & Development*, **23**(2-3): 123–129.  
715        <https://doi.org/10.1080/07924259.1993.9672303>
- 716        Maynard, A., Bible, J. M., Pespeni, M. H., Sanford, E., & Evans, T. G. (2018). Transcriptomic  
717        responses to extreme low salinity among locally adapted populations of Olympia oyster  
718        (*Ostrea lurida*). *Molecular Ecology*, **27**(21): 4225–4240. <https://doi.org/10.1111/mec.14863>
- 719        McGrath, T., McGovern, E., Gregory, C., & Cave, R. R. (2019). Local drivers of the seasonal  
720        carbonate cycle across four contrasting coastal systems. *Regional Studies in Marine  
721        Science*, **30**: 100733. <https://doi.org/10.1016/j.rsma.2019.100733>
- 722        McGraw, K. A. (2009). The Olympia Oyster, *Ostrea lurida* Carpenter 1864 Along the West  
723        Coast of North America. *Journal of Shellfish Research*, **28**(1): 5–10.  
724        <https://doi.org/10.2983/035.028.0110>
- 725        Moore, J. D., Marshman, B. C., Obernolte, R., & Abbott, R. (2016). Sexual development and  
726        symbionts of native Olympia oysters *Ostrea lurida* naturally settled on cultch deployed in  
727        San Francisco Bay, California. *California Fish and Game*, **102**(3): 100–118.  
728        <https://nrm.dfg.ca.gov/FileHandler.ashx?DocumentID=136509&inline> (accessed September  
729        24, 2019).
- 730        Oates, M. (2013). *Observations of gonad structure and gametogenic timing in a recovering*

- 731 population of *Ostrea lurida* (Carpenter 1864) (MS thesis). University of Oregon, Eugene,  
732 OR 66 pp.
- 733 Olson, C. E., & Roberts, S. B. (2015). Indication of family-specific DNA methylation patterns in  
734 developing oysters. *BioRxiv*. p. 012831. <https://doi.org/10.1101/012831>
- 735 Paaby, A. B., & Rockman, M. V. (2014). Cryptic genetic variation: evolution's hidden substrate.  
736 *Nature Reviews. Genetics*, **15**(4): 247–258. <https://doi.org/10.1038/nrg3688>
- 737 Parker, L. M., Ross, P. M., & O'Connor, W. A. (2011). Populations of the Sydney rock oyster,  
738 *Saccostrea glomerata*, vary in response to ocean acidification. *Marine Biology*, **158**(3): 689–  
739 697. <https://doi.org/10.1007/s00227-010-1592-4>
- 740 Parker, L. M., Ross, P. M., O'Connor, W. A., Borysko, L., Raftos, D. A., & Pörtner, H.O.  
741 (2012). Adult exposure influences offspring response to ocean acidification in oysters.  
742 *Global Change Biology*, **18**(1): 82–92. <https://doi.org/10.1111/j.1365-2486.2011.02520.x>
- 743 Parker, L. M., O'Connor, W. A., Raftos, D. A., Pörtner, H.O., & Ross, & P. M. (2015).  
744 Persistence of Positive Carryover Effects in the Oyster, *Saccostrea glomerata*, following  
745 Transgenerational Exposure to Ocean Acidification. *PLoS One*, **10**(7): e0132276.  
746 <https://doi.org/10.1371/journal.pone.0132276>
- 747 Parker, L. M., O'Connor, W. A., Byrne, M., Coleman, R. A., Virtue, P., Dove, M., Gibbs, M.,  
748 Spohr, L., Scanes, E., & Ross, P. M. (2017). Adult exposure to ocean acidification is  
749 maladaptive for larvae of the Sydney rock oyster *Saccostrea glomerata* in the presence of  
750 multiple stressors. *Biology Letters*, **13**: 20160798. <https://doi.org/10.1098/rsbl.2016.0798>
- 751 Parker, L. M., O'Connor, W. A., Byrne, M., Dove, M., Coleman, R. A., Pörtner, H.O., Scanes,  
752 E., Virtue, P., Gibbs, M., & Ross, P. M. (2018). Ocean acidification but not warming alters  
753 sex determination in the Sydney rock oyster, *Saccostrea glomerata*. *Proc. R. Soc. B*,

- 754           **285**(1872): 20172869. <https://doi.org/10.1098/rspb.2017.2869>
- 755       Pelletier, G., Roberts, M., Keyzers, M., & Alin, S. R. (2018). Seasonal variation in aragonite  
756       saturation in surface waters of Puget Sound – a pilot study. *Elementa: Science of the  
757       Anthropocene*, **6**(1): 5. <http://doi.org/10.1525/elementa.270>
- 758       Perez, M. F., & Lehner, B. (2019). Intergenerational and transgenerational epigenetic inheritance  
759       in animals. *Nature Cell Biology*, **21**(2): 143–151. [https://doi.org/10.1038/s41556-018-0242-9](https://doi.org/10.1038/s41556-018-0242-<br/>760       9)
- 761       Philippart, C. J. M., van Aken, H. M., Beukema, J. J., Bos, O. G., Cadée, G. C., & Dekker, R.  
762       (2003). Climate-related changes in recruitment of the bivalve Macoma balthica. *Limnology  
763       and Oceanography*, **48**(6): 2171–2185. <https://doi.org/10.4319/lo.2003.48.6.2171>
- 764       Polson, M. P., & Zacherl, D. C. (2009). Geographic Distribution and Intertidal Population Status  
765       for the Olympia Oyster, Ostrea lurida Carpenter 1864, from Alaska to Baja. *Journal of  
766       Shellfish Research*, **28**(1): 69–77. <https://doi.org/10.2983/035.028.0113>
- 767       Przeslawski, R., Byrne, M., & Mellin, C. (2015). A review and meta-analysis of the effects of  
768       multiple abiotic stressors on marine embryos and larvae. *Global Change Biology*, **21**(6):  
769       2122–2140. <https://doi.org/10.1111/gcb.12833>
- 770       Putnam, H. M., & Gates, R. D. (2015). Preconditioning in the reef-building coral Pocillopora  
771       damicornis and the potential for trans-generational acclimatization in coral larvae under  
772       future climate change conditions. *The Journal of Experimental Biology*, **218**(15): 2365–  
773       2372. <https://doi.org/10.1242/jeb.123018>
- 774       R Core Team. (2016). R: A language and environment for statistical computing (Version  
775       1.1.383). Retrieved from <https://www.R-project.org/>
- 776       Rodgers, A. B., Morgan, C. P., Bronson, S. L., Revello, S., & Bale, T. L. (2013). Paternal stress

- 777 exposure alters sperm microRNA content and reprograms offspring HPA stress axis  
778 regulation. *The Journal of Neuroscience: The Official Journal of the Society for*  
779 *Neuroscience*, **33**(21): 9003–9012. <https://doi.org/10.1523/JNEUROSCI.0914-13.2013>
- 780 Ross, P. M., Parker, L., & Byrne, M. (2016). Transgenerational responses of molluscs and  
781 echinoderms to changing ocean conditions. *ICES Journal of Marine Science: Journal Du*  
782 *Conseil*, **73**(3): 537–549. <https://doi.org/10.1093/icesjms/fsv254>
- 783 Sanford, E., & Kelly, M. W. (2011). Local adaptation in marine invertebrates. *Annual Review of*  
784 *Marine Science*, **3**: 509–535. <https://doi.org/10.1146/annurev-marine-120709-142756>
- 785 Santerre, C., Sourdaine, P., Marc, N., Mingant, C., Robert, R., & Martinez, A.-S. (2013). Oyster  
786 sex determination is influenced by temperature - first clues in spat during first gonadic  
787 differentiation and gametogenesis. *Comparative Biochemistry and Physiology. Part A,*  
788 *Molecular & Integrative Physiology*, **165**(1): 61–69.  
789 <https://doi.org/10.1016/j.cbpa.2013.02.007>
- 790 Silliman, K. (2019). Population structure, genetic connectivity, and adaptation in the Olympia  
791 oyster (*Ostrea lurida*) along the west coast of North America. *Evolutionary Applications*,  
792 **12**(5): 923-939. <https://doi.org/10.1111/eva.12766>
- 793 Silliman, K. E., Bowyer, T. K., & Roberts, S. B. (2018). Consistent differences in fitness traits  
794 across multiple generations of Olympia oysters. *Scientific Reports*, **8**(1): 6080.  
795 <https://doi.org/10.1038/s41598-018-24455-3>
- 796 Sokolova, I. M., Frederich, M., Bagwe, R., Lannig, G., & Sukhotin, A. A. (2012). Energy  
797 homeostasis as an integrative tool for assessing limits of environmental stress tolerance in  
798 aquatic invertebrates. *Marine Environmental Research*, **79**: 1–15.  
799 <https://doi.org/10.1016/j.marenvres.2012.04.003>

- 800 Soubry, A., Hoyo, C., Jirtle, R. L., & Murphy, S. K. (2014). A paternal environmental legacy:  
801 evidence for epigenetic inheritance through the male germ line. *BioEssays: News and*  
802 *Reviews in Molecular, Cellular and Developmental Biology*, **36**(4): 359–371.  
803 <https://doi.org/10.1002/bies.201300113>
- 804 Spencer, L. H., Y. R. Venkataraman, R. Crim, S. Ryan, M. J. Horwith, & S. B. Roberts.  
805 Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations.  
806 GitHub repository. <https://doi.org/10.6084/m9.figshare.8872646>.
- 807 Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H., & Reusch, T. B. H. (2014).  
808 Evolution in an acidifying ocean. *Trends in Ecology & Evolution*, **29**(2): 117–125.  
809 <https://doi.org/10.1016/j.tree.2013.11.001>
- 810 Thompson, E. L., O'Connor, W., Parker, L., Ross, P., & Raftos, D. A. (2015). Differential  
811 proteomic responses of selectively bred and wild-type Sydney rock oyster populations  
812 exposed to elevated CO<sub>2</sub>. *Molecular Ecology*, **24**(6): 1248–1262.  
813 <https://doi.org/10.1111/mec.13111>
- 814 Thomsen, J., Stapp, L. S., Haynert, K., Schade, H., Danelli, M., Lannig, G., Wegner, K. M., &  
815 Melzner, F. (2017). Naturally acidified habitat selects for ocean acidification-tolerant  
816 mussels. *Science Advances*, **3**(4): e1602411. <https://doi.org/10.1126/sciadv.1602411>
- 817 Thor, P., & Dupont, S. (2015). Transgenerational effects alleviate severe fecundity loss during  
818 ocean acidification in a ubiquitous planktonic copepod. *Global Change Biology*, **21**(6),  
819 2261–2271.
- 820 Utting, S. D., & Millican, P. F. (1997). Techniques for the hatchery conditioning of bivalve  
821 broodstocks and the subsequent effect on egg quality and larval viability. *Aquaculture*,  
822 **155**(1): 45–54. [https://doi.org/10.1016/S0044-8486\(97\)00108-7](https://doi.org/10.1016/S0044-8486(97)00108-7)

- 823 Venkataraman, Y. R., Spencer, L. H., & Roberts, S. B. (2019). Adult low pH exposure  
824 influences larval abundance in Pacific oysters (*Crassostrea gigas*). University of  
825 Washington ResearchWorks Archive, <http://hdl.handle.net/1773/43182>. Accepted and in  
826 press in *Journal of Shellfish Research*.
- 827 Waldbusser, G. G., Gray, M. W., Hales, B., Langdon, C. J., Haley, B. A., Gimenez, I., Smith, S.  
828 R., Brunner, E. L., & Hutchinson, G. (2016). Slow shell building, a possible trait for  
829 resistance to the effects of acute ocean acidification. *Limnology and Oceanography*, **61**(6):  
830 1969–1983. <https://doi.org/10.1002/lno.10348>
- 831 Wasson, K., Hughes, B. B., Berriman, J. S., Chang, A. L., Deck, A. K., Dinnel, P. A., Endris, C.,  
832 Espinoza, M., Dudas, S., Ferner, M. C., Grosholz, E. D., Kimbro, D., Ruesink, J. L.,  
833 Trimble, A. C., Vander Schaaf, D., Zabin, C. J., & Zacherl, D. C. (2016). Coast-Wide  
834 Recruitment Dynamics of Olympia Oysters Reveal Limited Synchrony and Multiple  
835 Predictors of Failure. *Ecology* **97**(12): 3503–16. <https://doi.org/10.1002/ecy.1602>
- 836 White, S. J., Vadopalas, B., Silliman, K., & Roberts, S. B. (2017). Genotype-by-sequencing of  
837 three geographically distinct populations of Olympia oysters, *Ostrea lurida*. *Scientific Data*,  
838 **4**: 170130. <https://doi.org/10.1038/sdata.2017.130>
- 839 Wickham, H. (2017). ggplot2 - Elegant Graphics for Data Analysis (2nd Edition). *Journal of  
840 Statistical Software, Book Reviews*, **77**(2): 1–3. <https://doi.org/10.18637/jss.v077.b02>
- 841 Wilson, J. A., Chaparro, O. R., & Thompson, R. J. (1996). The importance of broodstock  
842 nutrition on the viability of larvae and spat in the Chilean oyster *Ostrea chilensis*.  
843 *Aquaculture*, **139**(1): 63–75. [https://doi.org/10.1016/0044-8486\(95\)01159-5](https://doi.org/10.1016/0044-8486(95)01159-5)

**Table 1: Environmental data during offspring field trial.** Environmental data

was collected from locations where offspring were deployed for 3 months from June through August 2018. Mean $\pm$ SD of continuously monitored environmental data are shown for periods of tidal submergence only (tidal height  $>0.3\text{m}$ ), collected at two deployment locations within each bay.

	Fidalgo Bay	Port Gamble Bay	Skokomish River Delta	Case Inlet
<b>Temperature (°C)</b>	15.4 $\pm$ 1.5	15.0 $\pm$ 1.0	16.2 $\pm$ 2.7	16.8 $\pm$ 1.7
<b>DO (mg/L)</b>	10.6 $\pm$ 2.4	10.5 $\pm$ 1.9	10.2 $\pm$ 3.9	11.2 $\pm$ 2.8
<b>Salinity (PSU)</b>	28.5 $\pm$ 3.9	31.9 $\pm$ 2.0	29.6 $\pm$ 1.3	24.6 $\pm$ 1.7
<b>pH<sub>T</sub></b>	8.07 $\pm$ 0.15	7.86 $\pm$ 0.17	8.01 $\pm$ 0.20	8.01 $\pm$ 0.16
<b>Chlorophyll (μg/L)</b>	2.27 $\pm$ 4.09	2.25 $\pm$ 1.45	5.72 $\pm$ 15.36	3.31 $\pm$ 6.13

**Table 2: Gonad stage and sex comparisons among treatments.** Gonad was sampled after temperature treatment but before pCO<sub>2</sub> (6°C Pre and 10°C Pre, n=54), and after pCO<sub>2</sub> treatment (Amb=841±85 µatm, n=39; High= 3045±488 µatm, n=39). Pearson's chi-square statistics are shown with p-adj in parentheses for gonad sex, stage of the dominant sex, male gametes when present, and female gametes when present. Cells with \* and in bold indicate significant differences between comparison; blank cells=not tested; % of mature = % of sampled oysters that contained stage 3 male or female gametes, per treatment.

Temperature		6°C			10°C			6°C			10°C		
	pCO <sub>2</sub>	Pre	Amb	High	Pre	Amb	High	Pre	Amb	High	Pre	Amb	High
6°C	Pre	-											
	Amb	0.8 (0.93)	-										
	High	4.6 (0.34)	5.4 (0.29)	-									
10°C	Pre	5.9 (0.26)				-							
	Amb				6.8 (0.18)	-							
	High		5.3 (0.29)		3.8 (0.46)	0.6 (0.94)	-						
<i>Sex Ratio</i>													
6°C	Pre	-											
	Amb	*16.5 (0.013)	-										
	High	4.6 (0.48)	9.7 (0.090)	-									
10°C	Pre	*15.8 (0.017)				-							
	Amb				*12.7 (0.038)	-							
	High		2.8 (0.78)		5.2 (0.44)	*12.5 (0.038)	-						
<i>Stage of the dominant sex</i>													
6°C	Pre	-											
	Amb	*24.2 (1.6e-3)	-										
	High	*15.2 (0.013)	9.0 (0.071)	-									
10°C	Pre	*31.1 (1.6e-3)				-							
	Amb				*11.2 (0.038)	-							
	High		1.7 (0.78)		0.6 (0.95)	9.5 (0.084)	-						
<b>% mature</b>		30%	28%	15%	19%	33%	21%						
<i>Male gametes</i>													
6°C	Pre	-											
	Amb	6.3 (0.18)	-										
	High	3.6 (0.47)	4.4 (0.36)	-									
10°C	Pre	2.1 (0.78)				-							
	Amb				4.2 (0.26)	-							
	High		0.8 (0.9)		5.5 (0.17)	0.15 (1.0)	-						
<i>Female gametes</i>													
<b>% mature</b>		2%	15%	8%	6%	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 x bay x parental pCO<sub>2</sub> treatment (Amb=841 $\pm$ 85  $\mu$ atm, High= 3045 $\pm$ 488  $\mu$ atm). Only offspring from 6°C-treated adults were deployed. Significant survival differences were detected between parental pCO<sub>2</sub> treatment within the Fidalgo Bay and Oyster Bay F2 cohorts (\*), and across all cohorts (+).

Cohort →	Fidalgo Bay (F)		Dabob Bay (D)		Oyster Bay F1 (O-1)		Oyster Bay F2 (O-2)		All cohorts	
pCO <sub>2</sub> → Bay ↓	Amb	High	Amb	High	Amb	High	Amb	High	Amb	High
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%
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%

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

848 **Figure 2:** Experimental timeline. Four cohorts of adult *O. lurida* (F, D, O-1, O-2) were  
849 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<sub>2</sub> levels  
850 ( $841 \pm 85 \mu\text{atm}$ ,  $3045 \pm 488 \mu\text{atm}$ ). They were returned to ambient pCO<sub>2</sub> conditions to volitionally  
851 spawn. Larvae were collected and reared by cohort x temperature x pCO<sub>2</sub>. Juveniles (~1 year)  
852 from 6°C-Ambient pCO<sub>2</sub> and 6°C-Low pCO<sub>2</sub> adults were deployed in 4 bays in Puget Sound.

853 **Figure 3:** Gonad developmental stages for male and female gametes, after 60-days in  
854 temperature treatments but before pCO<sub>2</sub> treatments ("Pre", n=54) and after 52 days in high pCO<sub>2</sub>  
855 ( $3045 \pm 488 \mu\text{atm}$ , n=39) and ambient pCO<sub>2</sub> ( $841 \pm 85 \mu\text{atm}$ , n=39), which indicates that sperm  
856 development was influenced by elevated winter temperature (more advanced) and high pCO<sub>2</sub>  
857 (less advanced, 10°C treatment only), but oocyte development was not. All oysters were  
858 assigned both male & female stages; if no oocytes were present, for example, that oyster was  
859 designated as female stage 0.

860 **Figure 4:** Gonad sex, after 60-days in temperature treatments but before pCO<sub>2</sub> treatments ("Pre",  
861 n=54) and after 52 days in high pCO<sub>2</sub> ( $3045 \pm 488 \mu\text{atm}$ , n=39) and ambient pCO<sub>2</sub> ( $841 \pm 85 \mu\text{atm}$ ,  
862 n=39). Winter conditions did not significantly influence gonad sex ratios.

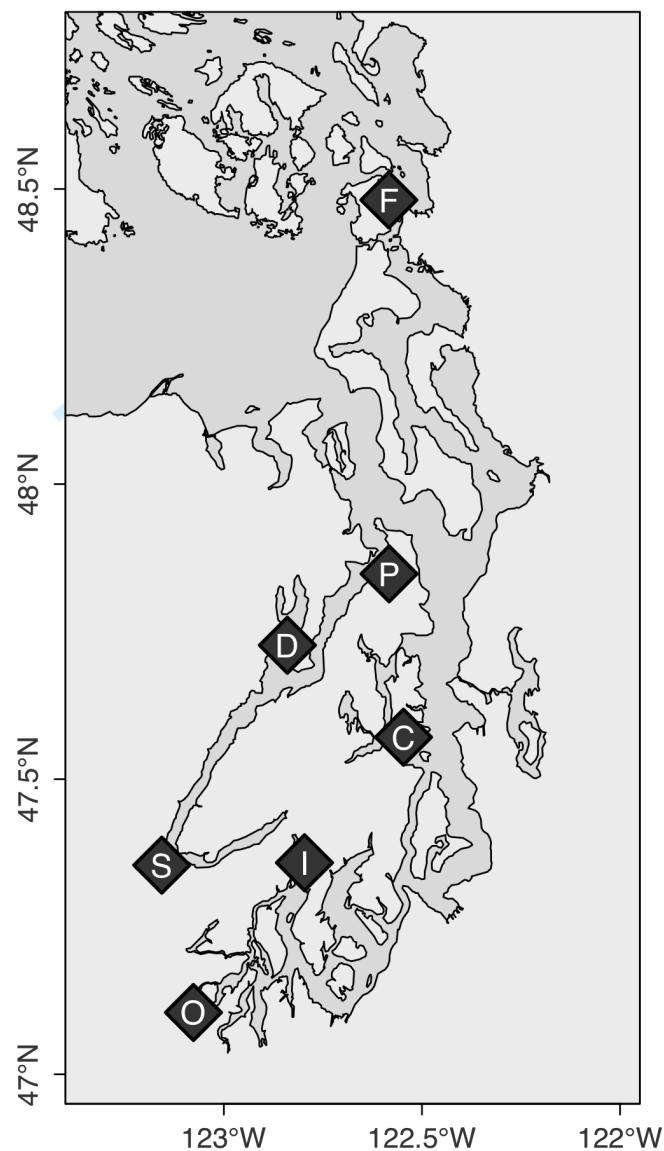
863 **Figure 5:** Cumulative larvae released over 90 days of continuous volitional spawning under  
864 hatchery conditions, normalized by the number of adult oysters. Each of the four panels represent  
865 a cohort, and lines are color coded by winter temperature and pCO<sub>2</sub> treatments, where ambient

866  $pCO_2 = 841 \mu\text{atm}$  (7.8 pH), and high  $pCO_2 = 3045 \mu\text{atm}$  (7.31). Reproductive conditioning and  
867 spawning occurred at  $18^\circ\text{C}$ , in ambient  $pCO_2$ , and with live algae at a density of  $66,000 \pm 12,000$   
868 cells/mL.

869 **Figure 6:** Left: average number of larvae collected on a daily basis (excluding days where no  
870 larvae were released). Daily pulses of larvae were larger in  $10^\circ\text{C}$  than  $6^\circ\text{C}$ , but only in oysters  
871 exposed to ambient  $pCO_2$ . For statistical analysis, data was normalized by number of oysters \*  
872 average oyster height (cm) (data shown is not normalized). Right: number of spawning days until  
873 larval release peaked; peak release occurred on average 8.3 days earlier in  $10^\circ\text{C}$  treated oysters.  
874 Letters (a, ab, b) indicate differences among treatments. Boxes contain values lying within the  
875 interquartile range (IQR), with medians indicated by lines in the middle of boxes. Whiskers  
876 extend to the largest value no greater than 1.5\*IQR.

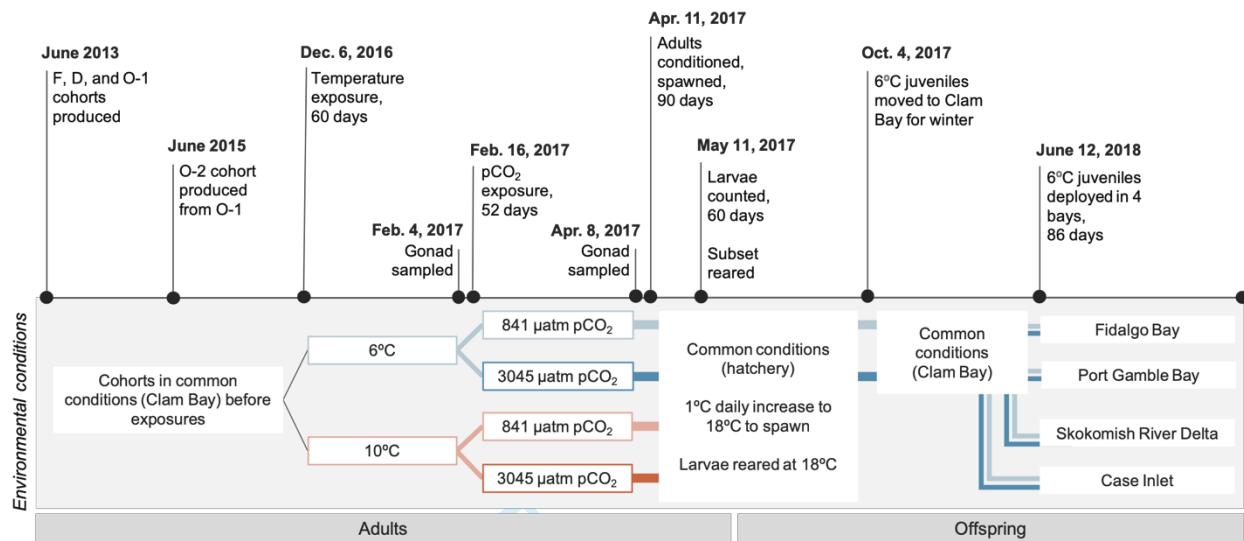
877 **Figure 7:** Percent survival of juvenile offspring in the field. The four panels each represent  
878 survival in one bay (Fidalgo Bay, Port Gamble Bay, Skokomish River Delta, Case Inlet). Within  
879 each panel, boxplots are separated by parental  $pCO_2$  exposure (Ambient=841  $\mu\text{atm}$ , High=3045  
880  $\mu\text{atm}$ ). Points indicate % survival in each deployment pouch, and symbols indicate cohort  
881 (Fidalgo Bay, Dabob Bay, Oyster Bay Cohort 1, and Oyster Bay Cohort 2). Letters (a, b) indicate  
882 survival differences among parental  $pCO_2$  exposure within each bay. Boxes contain values lying  
883 within the interquartile range (IQR), with median survival indicated by lines in the middle of  
884 boxes. Whiskers extend to the largest value no greater than 1.5\*IQR.

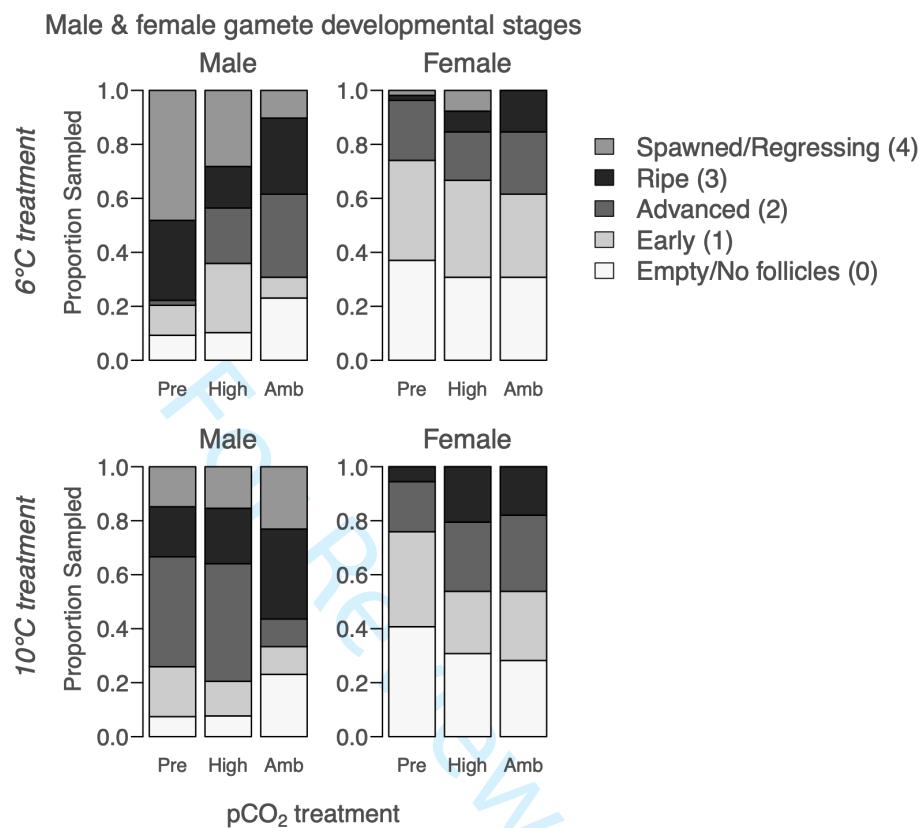
885

**Figure 1**

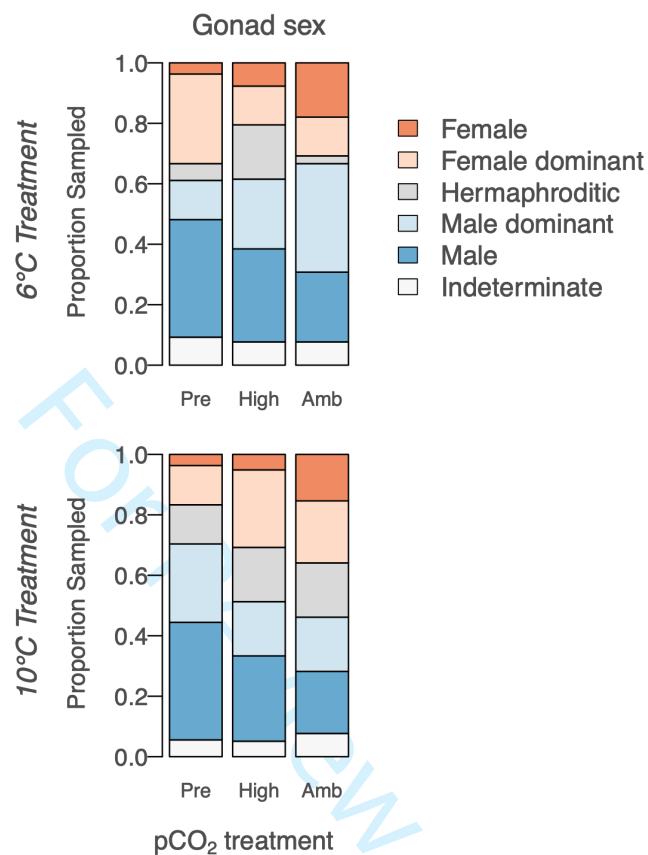
886

Figure 2

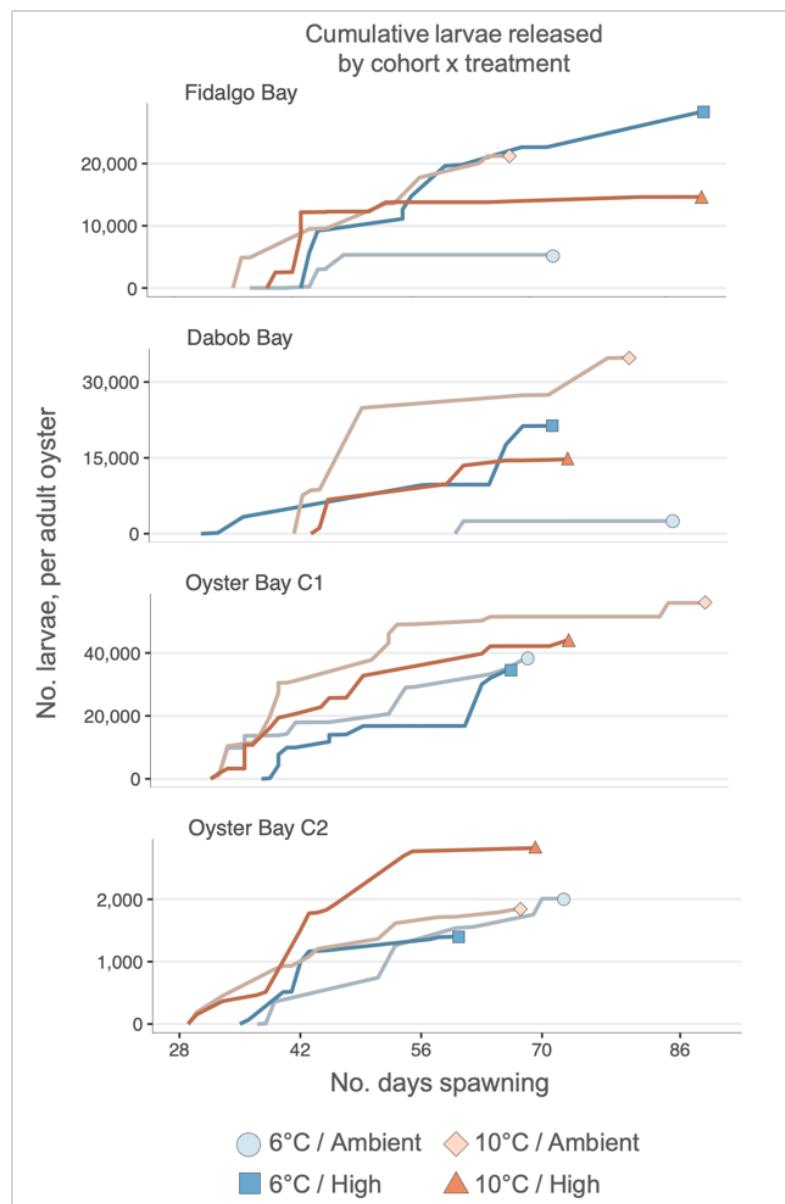


**Figure 3**

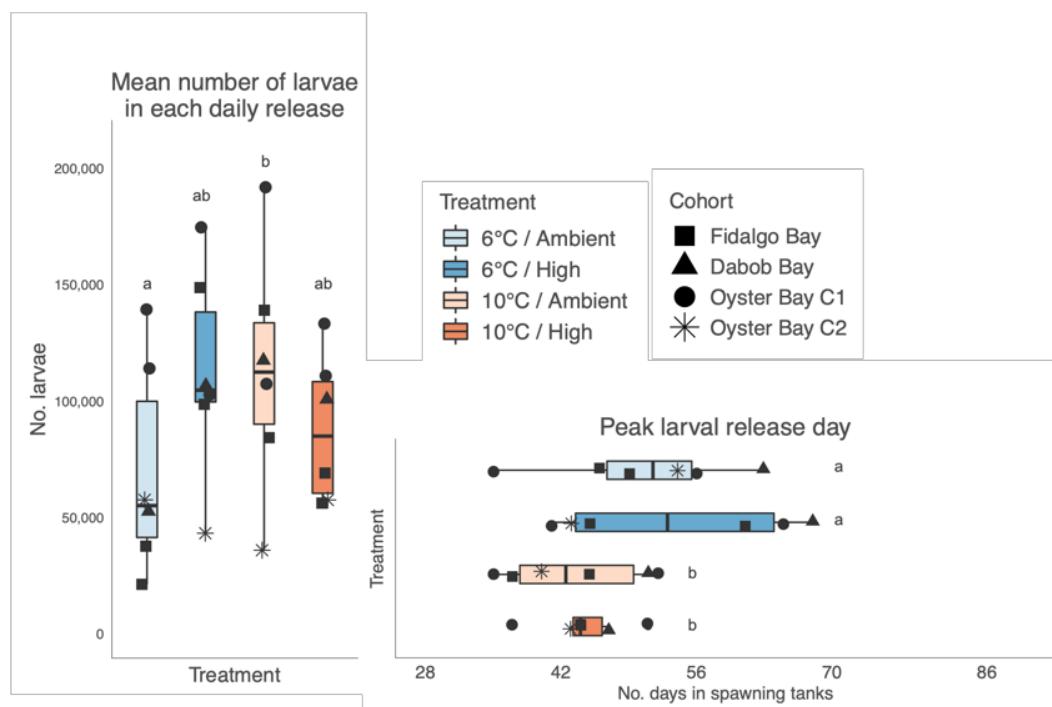
888

**Figure 4**

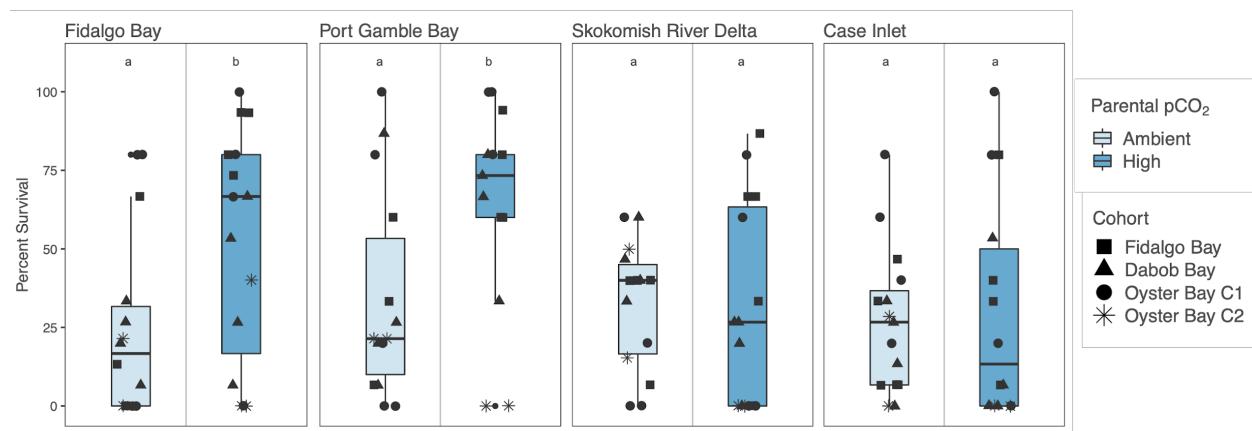
889

**Figure 5**

890

**Figure 6**

891

**Figure 7**

## Appendix S1

Spencer, L. H., Y. R. Venkataraman, R. Crim, S. Ryan, M. J. Horwith, & S. B. Roberts. **Carryover effects of temperature and pCO<sub>2</sub> across multiple Olympia oyster populations.** *Ecological Applications*.

Data, R script analyses, gonad tissue images, and high-resolution supplementary figures are available in the associated GitHub repository: <https://doi.org/10.6084/m9.figshare.8872646>

### Section S1: 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 MgSO<sub>4</sub>-(H<sub>2</sub>O)<sub>7</sub> (Fisher Scientific)
- 0.0107 mol of CaCl<sub>2</sub>-2(H<sub>2</sub>O) (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

### Section S2: Instruments used in experimental treatments in the hatchery

- Temperature treatment manipulation and monitoring:
  - o Chilling experimental water: Teco Aquarium Chiller, Model TK-500
  - o Continuous temperature measurements: Onset HOBO Water Temperature Data Loggers, Model U22-001
- pCO<sub>2</sub> treatment manipulation and monitoring:
  - o pH monitoring: Durafet pH probes, Honeywell Model 51453503-505
  - o pCO<sub>2</sub> injection control: Honeywell Dual Input Analytical Analyzer, Model 50003691-501
  - o Temperature monitoring: HOBO Pendant Temperature Data Loggers, Model UA-002-64
- Carbonate chemistry measurements:
  - o Temperature: Fisher Traceable Digital Thermometer, Model 15-077
  - o Salinity: VWR Bench/Portable Conductivity Meter, Model 23226-505
  - o pH: Mettler Toledo Combination pH Electrode, Model 11278-220
  - o Total alkalinity: Mettler Toledo Excellence Titrator, Model T5 Rondolino
- Algal dosing: metering pump – Iwaki EZ Controller, Model EZCD1

### Instruments used in field trial for continuous environmental data collection:

- 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
- Chlorophyll: Turner Designs Cyclops-7F Submersible Sensor with PME Cyclops-7 Data Loggers

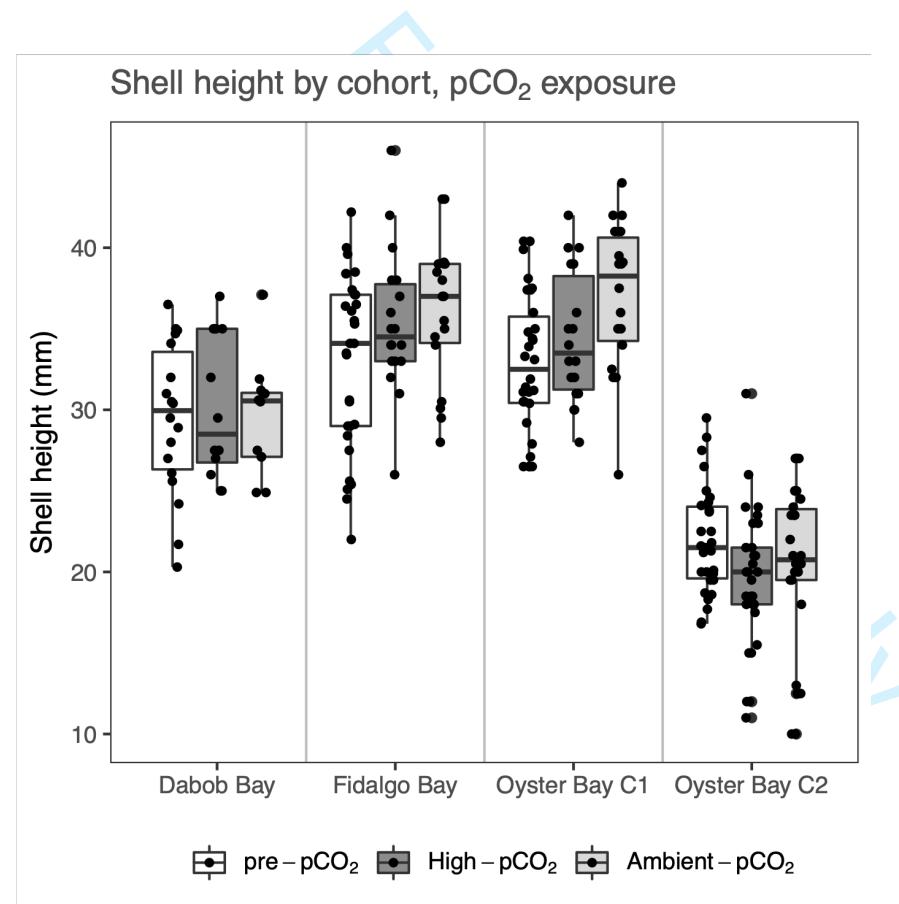
**Table S1:** Carbonate chemistry parameters for three time points during the pCO<sub>2</sub> treatments, which are averages ( $\pm$  SE) from three replicate tanks per treatment. All parameters except for total alkalinity differed significantly between control/ambient (Amb.) and experimental/high (High.) tanks (One-way ANOVA). More details are available in Venkataraman, Spencer & Roberts, 2019.

Day	pH***		Total Alkalinity ( $\mu\text{mol/kg}$ )		pCO <sub>2</sub> ( $\mu\text{atm}$ )***		DIC ( $\mu\text{mol/kg}$ )*		$\Omega_{\text{calcite}}$ ***		$\Omega_{\text{aragonite}}$ ***	
	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

### Section S3: 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 pCO<sub>2</sub> 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 pCO<sub>2</sub> treatments, adult shell height did not vary between temperatures treatments, but did among F1 cohorts, with D smaller than F ( $p=0.043$ ). After pCO<sub>2</sub> treatment, D was smaller than both F ( $p=3.4\text{e-}6$ ) and O-1 ( $p=3.5\text{e-}6$ ). The O-1 cohort increased in size ( $p=0.019$ ) in ambient pCO<sub>2</sub>, but not in high pCO<sub>2</sub>. No size differences among pre-pCO<sub>2</sub> and post-pCO<sub>2</sub> treatments were observed in the F, D or O-2 cohorts.



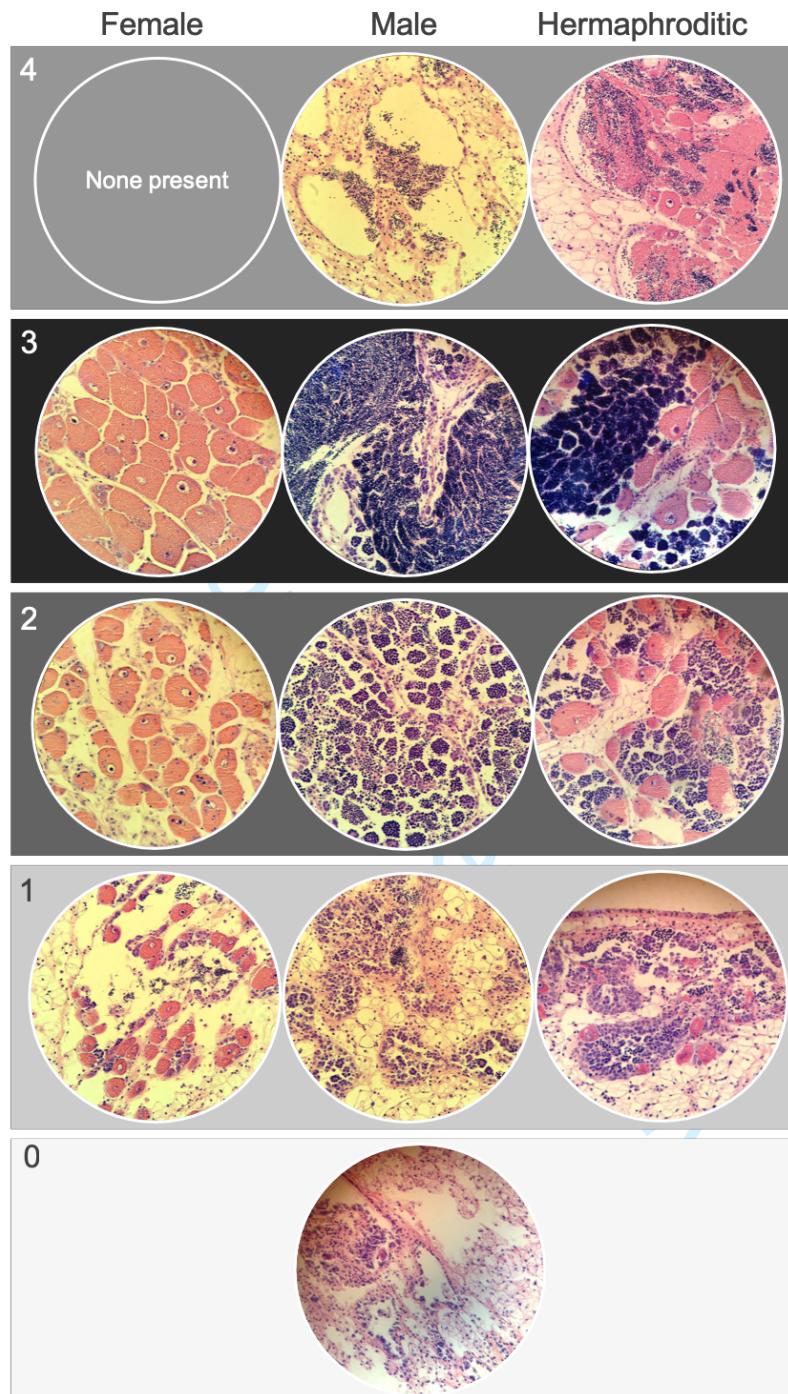
**Figure S1:** Adult shell height for each cohort, after temperature treatment but before pCO<sub>2</sub> treatment (“Pre-pCO<sub>2</sub>”), and after 52 days in High-pCO<sub>2</sub> ( $3045\pm488$   $\mu\text{atm}$ , n=39), and Ambient-pCO<sub>2</sub> ( $7.82\pm0.02$ , n=39).

**Table S2:** 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.

**Table S3:** Gonad sex designations, from da Silva 2009

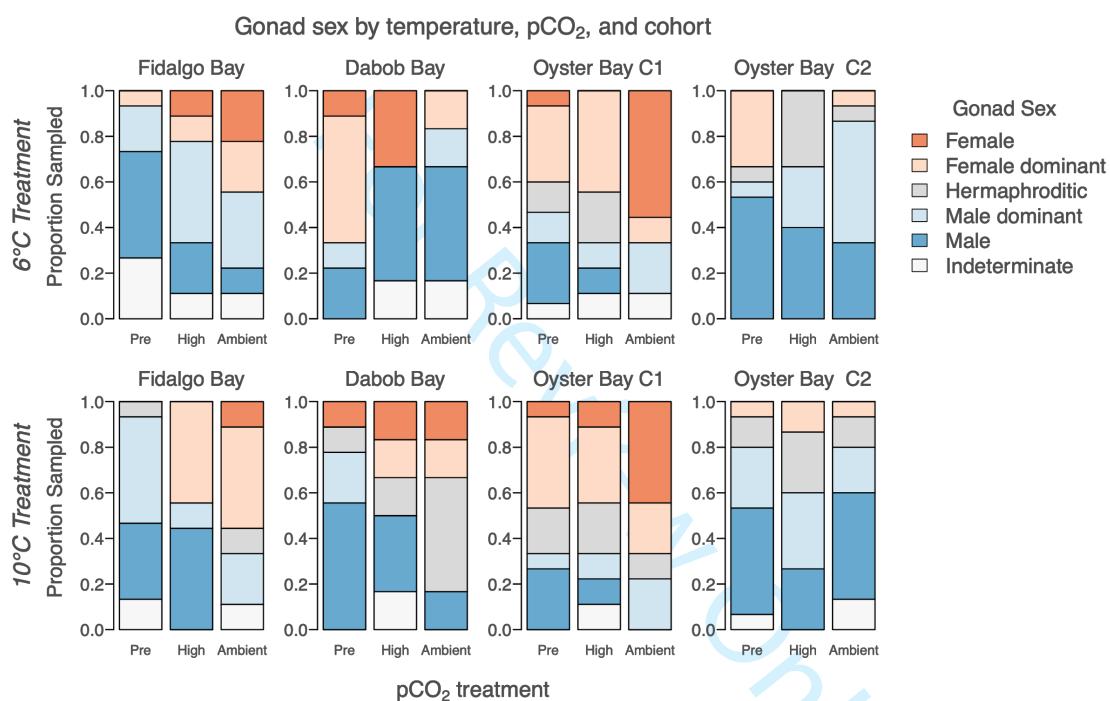
Sex (uncollapsed, from da Silva)	Sex, collapsed for statistical analyses	Designation	Description
F	F	Female	Follicles contain only female gonad material (any stage)
HPF	F	Hermaphroditic, predominantly female	Follicles contain predominantly female but also some male gonad material
H	H	Hermaphroditic	Follicles 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 gonia are visible



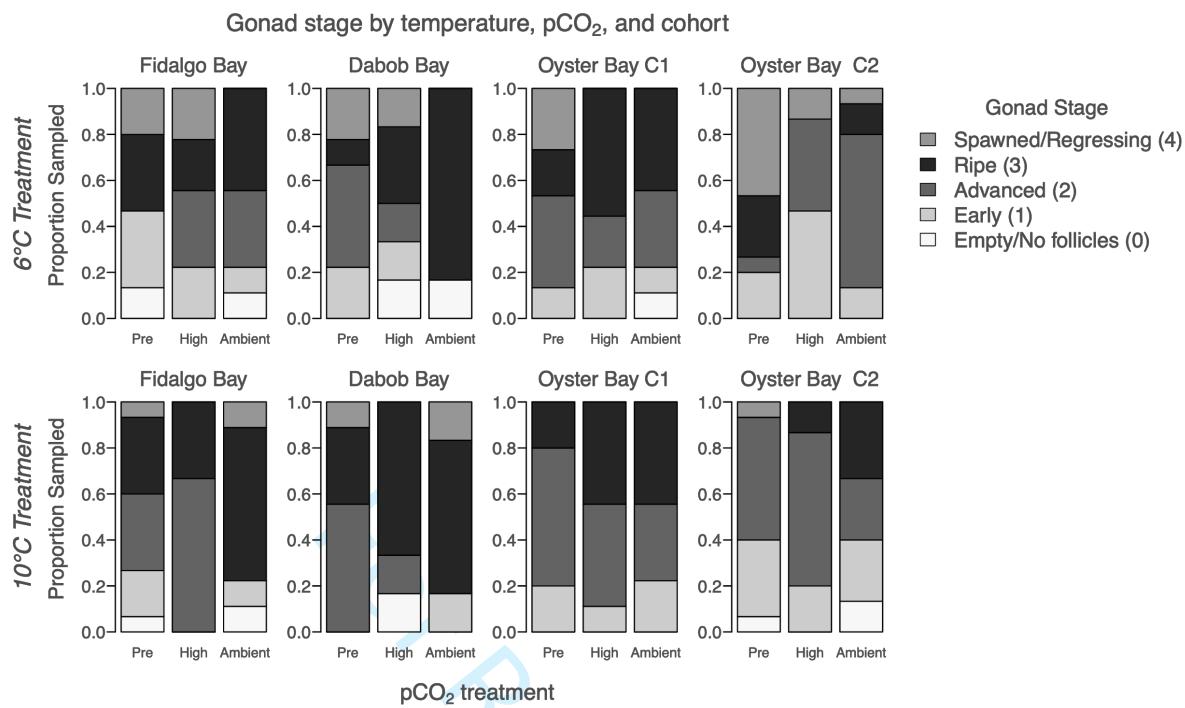
**Figure S2:** Examples of *Ostrea lurida* gonad stage designations. Stage 0 (no activity/sex differentiation); Stage 1 (early gametogenesis); Stage 2 (advanced gametogenesis); Stage 3 (late gametogenesis / ripe); Stage 4 (spawned and/or resorbing).

## Section S4: 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.



**Figure S3:** Gonad sex for each cohort, after temperature treatment but before pCO<sub>2</sub> treatment (“Pre”), and after 52 days in high pCO<sub>2</sub> (3045±488 µatm, n=39, “High”), and ambient pCO<sub>2</sub> (7.82±0.02, n=39, “Ambient”), separated by temperature treatment (6°C and 10°C).



**Figure S4:** Gonad stage of the dominant sex for each cohort, after temperature treatment but before pCO<sub>2</sub> treatment (“Pre”), and after 52 days in high pCO<sub>2</sub> ( $3045\pm488$   $\mu\text{atm}$ ,  $n=39$ , “High”), and ambient pCO<sub>2</sub> ( $7.82\pm0.02$ ,  $n=39$ , “Ambient”), separated by temperature treatment (6°C and 10°C).

**Table S4:** Frequency of dominant gonad stages by cohort and pCO<sub>2</sub> exposure, separated by winter temperature treatment (6°C, 10°C). Results of Chi-Square between pCO<sub>2</sub> 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 pCO<sub>2</sub> treatments. Stage differences were detected between pre-treatment (pre-pCO<sub>2</sub>) and after treatment for ambient and high pCO<sub>2</sub> separately, and are indicated by superscripts A and B: A= pre-treatment and ambient pCO<sub>2</sub> differed; B=pre-treatment and high pCO<sub>2</sub> differed ( $p<0.05$ ). Stage and sex comparisons in bold indicate that they were significantly different between temperature treatments, prior to pCO<sub>2</sub> treatments (“Pre” columns).

Dominant gonad stage for 6°C treatment, by cohort and pCO <sub>2</sub> exposure																
	Fidalgo Bay			Dabob Bay			Oyster Bay C1			Oyster Bay C2			All cohorts			
Gonad Stage	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	
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	
Total Sampled	15	9	9	9	6	6	15	9	9	15	15	15	54	39	39	
Dominant gonad stage for 10°C treatment, by cohort and pCO <sub>2</sub> exposure																
	Fidalgo Bay			Dabob Bay			Oyster Bay C1			Oyster Bay C2			All cohorts			
Gonad Stage	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	Pre pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	
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	
Total Sampled	15	9	9	9	6	6	15	9	9	15	15	15	54	39	39	

**Section S5: 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  $6.4\pm2.3$ ,  $8.0\pm2.9$ ,  $3.8\pm1.9$ , and  $2.8\pm1.0$  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).

For Review Only

**Table S5:** Timing and magnitude of larval production in 4 *Ostrea lurida* cohorts previously exposed to different winter temperatures (6°C and 10°C), then pCO<sub>2</sub> 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 <sub>2</sub> treatment		Amb. pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb. pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb. pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb. pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb. pCO <sub>2</sub>	High pCO <sub>2</sub>
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 larvae released (x10 <sup>3</sup> )	6°C	21/38	99/127	53	107	139/114	103/175	58	43	Ave: 79	Ave: 110
	10°C	139/84	58/56	117	101	192/107	133/111	36	57	Ave: 108	Ave: 88
Total larvae released (x10 <sup>3</sup> )	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 <sup>3</sup> )	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 <sup>3</sup> )	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

## Section S6: Larval rearing methods and survival

### 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 ( $\lesssim 50,000$ ) 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 pCO<sub>2</sub> 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 um 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 pCO<sub>2</sub>, affixed to the inside of shellfish cages, and hung in Clam Bay until June 2018.

<b>Table S6:</b> Tank densities during offspring rearing.											
		<i>Fidalgo Bay</i> (2 replicates)		<i>Dabob Bay</i>		<i>Oyster Bay - F1</i> (2 replicates)		<i>Oyster Bay - F2</i>		<i>All</i>	
		Amb pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	High pCO <sub>2</sub>	Amb pCO <sub>2</sub>	High pCO <sub>2</sub>
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 pCO<sub>2</sub> 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 pCO<sub>2</sub> 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 pCO<sub>2</sub>. 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 pCO<sub>2</sub> or temperature, but did differ by cohort ( $F(3,230)=5.73$ ,  $p=8.5e-4$ ). Pairwise tests indicate that O-1 survival was significantly lower than D ( $p=3.8e-4$ ), O-2 ( $p=5.4e-4$ ), and F ( $p=0.019$ ). Mean biweekly survival of D, F, O-2, and O-1 cohorts was 62±22%, 59±24%, 55±24%, and 49±28%, 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 pCO<sub>2</sub> ( $F(1,14)=1.9$ ,  $p=0.19$ ), 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 pCO<sub>2</sub> 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±71,100, 54,400±424000, 47,000±46,200, and 43,500±42,700, 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.

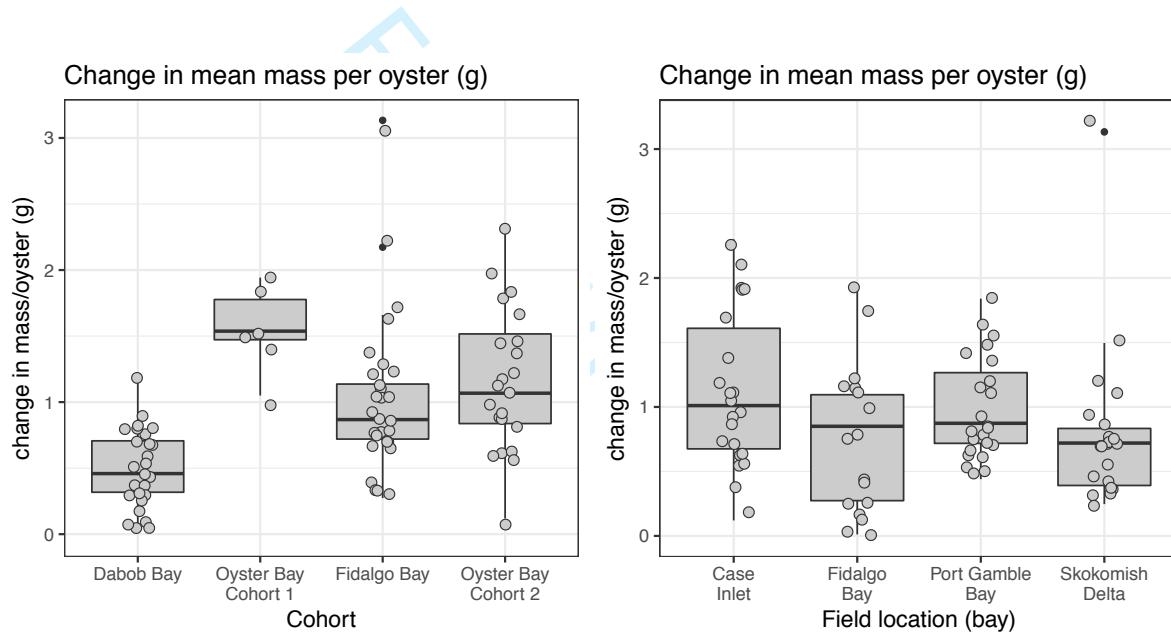
The number of days between first and last larval collection, and first and last eyed larvae varied by cohort, although this was not significant. Across treatments, eyed larvae were present soonest in F ( $14.5 \pm 2.5$  days), followed by O-1 ( $16.5 \pm 1.75$  days), O-2 ( $17.25 \pm 1.25$  days), and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

**Table S7:** Larval survival estimates by parental treatment and cohort.

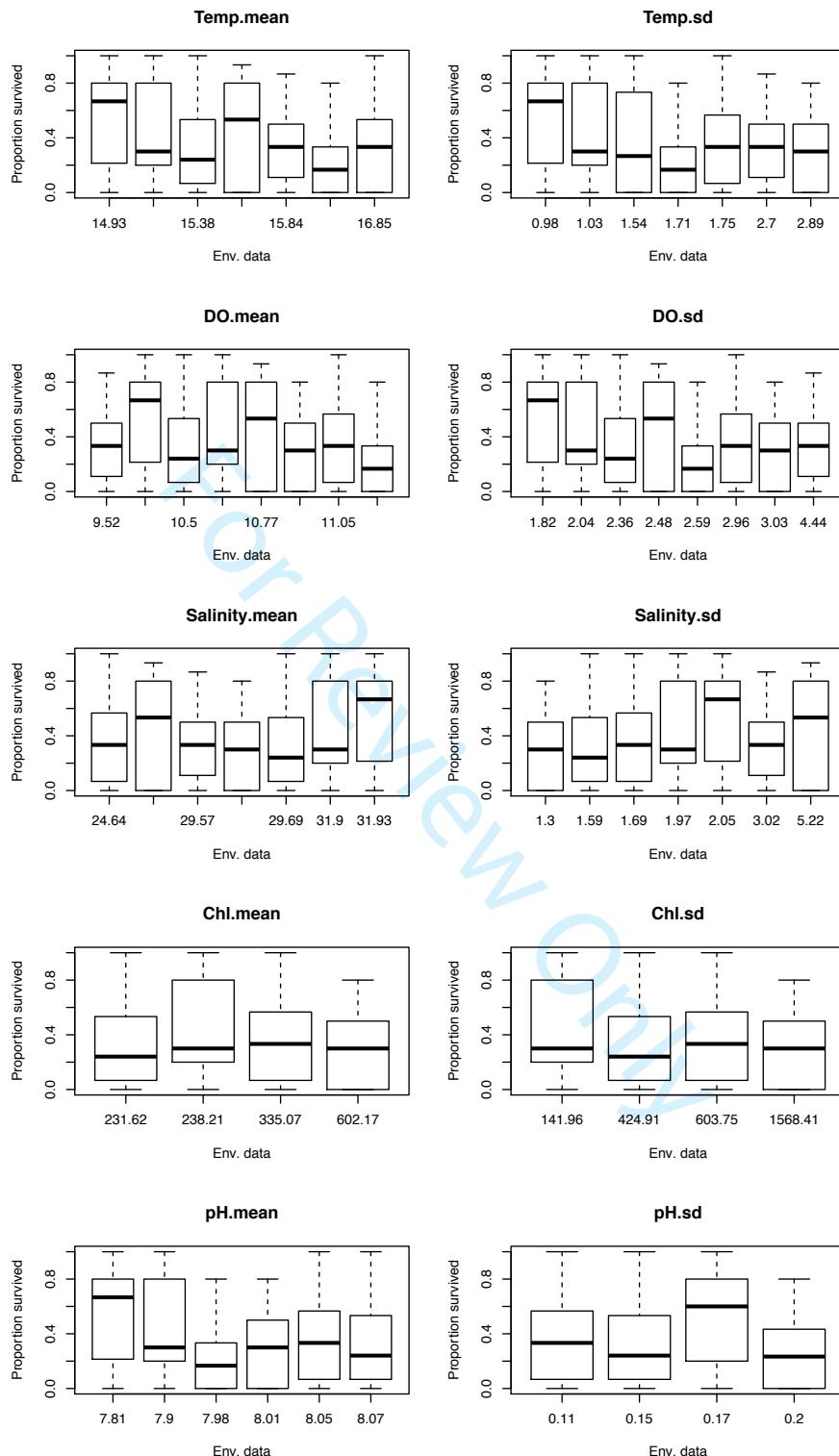
Larval survival, by treatment and cohort											
		Fidalgo Bay		Dabob Bay		Oyster Bay - F1		Oyster Bay - F2		All cohorts	
		Amb pCO <sub>2</sub>	High pCO <sub>2</sub>								
Average biweekly larval survival	6°C	56±25%	62±26%	69±18%	49±21%	44±23%	52±27%	60±24%	59±28%	56±24%	56±26%
	10°C	50±18%	51±26%	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%

## Section S7: Juvenile survival and environmental data

Associations among juvenile oyster survival and environmental summary statistics were explored to evaluate factors that best explain spatial variation in oyster survival. The mean and standard deviation of each environmental variable (temperature, dissolved oxygen, salinity, chlorophyll, and pH) during deployment were assessed independently by binomial generalized linear mixed models (glmm) using glmer from the lme4 package (vs. 1.1-19), and Wald tests with type II error. Significant single-factor variables were included in a full model, then backwards deletion was used to identify significant environmental factors in the most parsimonious model. Significant variables predicting juvenile survival included mean temperature, mean pH, and dissolved oxygen standard deviation. Figure S6 show proportion survival by each environmental summary statistic. See the “07\_Juvenile-deployment.R” R code in the Spencer et al. 2019 GitHub repository for analysis details and results.



**Figure S5:** Juvenile mass change during field trial was significantly less in the Dabob Bay cohort than other 3 cohorts (left), and was significantly less in Fidalgo Bay than in Port Gamble Bay and Case Inlet (right). Mean mass / oyster represents the average final mass per oyster minus average initial mass within each deployment bag.



**Figure S6:** Juvenile proportion survival ~ environmental summary statistics. Model selection using backwards deletion indicates that survival was significantly related to mean temperature (“Temp.mean”), mean pH (“pH.mean”), and dissolved oxygen standard deviation (“DO.sd”).