Responses to reviewer comments for "Carry-over effects of temperature and pCO2 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, i**n 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_2 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_2 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. T**he 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_2 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 pCO2 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 pCO2 and tempeatures 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_2 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 pCO2-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_2. 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_2), 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_2 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 (6ºC treatments) were tested during the field deployment.

COMMENT: (2) Please explain why only the pCO2 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_2) 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_2, only offspring from 6ºC 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.
