

SCHOOL OF AQUATIC AND FISHERY SCIENCES

COLLEGE OF THE ENVIRONMENT UNIVERSITY of WASHINGTON

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Dear Dr. Schimel,

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

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

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

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

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

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

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

Thank you for your consideration.

Sincerely, Laura H Spencer & Steven B Roberts School of Aquatic and Fishery Sciences University of Washington Seattle, WA 98105