

June 30, 2017

Dear Dr. Gargano,

We are pleased to provide the revised manuscript “Evidence of *Ostrea lurida* (Carpenter 1864) population structure in Puget Sound, WA” for publication in Marine Ecology. Thank you for providing the valuable reviews that we believe greatly improved the revised manuscript. We have made changes as suggested; our specific responses to each reviewer comment are outlined below.

Sincerely,



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Reviewer 1.

Remove the parentheses from the taxonomic authority in the title – *lurida* was originally described in *Ostrea*.

Response: removed parentheses

Use of &/and is inconsistent through the document, particularly in references to Figures and literature cited.

Response: changed to ‘&’ for consistency

In references to Figures, use of Figure and Fig. is inconsistent.

Response: changed ‘Fig.’ to ‘Figure’

Be consistent in the use of site names or designations; use gazetteer names for broodstock sources and Southern/Central/Northern/Hood Canal for outplant locations. Interchangeable use of names makes the document more difficult to read, not simpler.

Response: double checked text for consistency

Bible and Sanford (2016) is cited in the document (lines 57, 62, 144, 297) but not listed in the references.

Response: added reference to list (current version: lines 58, 63, 147, 303)

Refer to Figure 1 in the Bays of Origin section beginning on line 83. On line 114, delete “and oysters were removed from mesh bags” from the first sentence; this is covered in the subsequent sentence.

Response: removed phrase as suggested (current version: lines 84 and 117 respectively)

Consider adding buoy locations from the Environmental Monitoring section (starting on line 118) to Figure 1.

Response: The addition of buoy locations would render Figure 1 less readable due to close proximity to site designations; we therefore retained the specific location information in the text. (current version: line 121)

Wickham (2014) is cited in the document (line 122) but both Wickham (2011) and Wickham (2014) are listed in the references; check the date for the citation and remove the other reference from the list.

Response: removed Wickham (2014) from reference list. (current version: line 125)

On lines 160-161, change “Singmann, Bolker and Walker, 2015” to “Singmann et al., 2015”.

Response: changed as suggested (current version: line 163)

On line 172, change “H20” to “H₂O”.

Response: corrected typo (current version: line 175)

On line 211, insert actual numbers rather than placeholders for mortality of Oyster Bay sourced oysters outplanted at the Southern site.

Response: inserted correct numbers (current version: line 215)

Re-order figures or re-visit text in the sections on Growth (starting line 215) and Brooding Females (starting line 230) so that Figures are cited in numerical sequence.

Response: changed as suggested. Section now reads “At the end of the experiment the sizes of oysters among sites were significantly different (LME $F=268.29$, $df=2$, $P<0.0001$ & Kruskal-Wallis, $X^2=383.4$, $df=2$, $P<0.0001$), with the Southern site producing the largest oysters (Figures 5 & 6) and Central site producing the smallest (Figures 7 & 8). Oyster size also differed among populations ((LME $F=86.42$, $df=2$, $P=0.007$ & Kruskal-Wallis, $X^2=196.1$, $df=2$, $P<0.0001$). The linear model also indicated that the interaction between populations and sites was significant (LME $F=23.34$, $df=4$, $P<0.0001$). At the Southern site, Fidalgo Bay oysters were larger than Dabob (Nemenyi Post-Hoc, $P=<0.0001$) and

Oyster Bay oysters (Nemenyi Post-Hoc, $P < 0.0001$) (Figures 5 & 6). At the Central site, the Oyster Bay oysters were significantly larger than the Dabob oysters by the end of the experiment (Nemenyi Post-Hoc, $P = 0.00028$) (Figures 7 & 8). At the Northern site, oysters from Dabob Bay broodstock were smaller than Fidalgo Bay (Nemenyi Post-Hoc, $P < 0.0001$) and Oyster Bay (Nemenyi Post-Hoc, $P < 0.0001$) oysters at the end of the experiment (Figures 9 & 10). Oyster mean size at outplant was 11.4 (± 3.2 SD) mm and with no differences in size among population except for the Central site where the Fidalgo population was larger (Figure 8).” (current version: lines 218 and 232 respectively)

On line 274, change “referred to from here on as populations for simplicity” to “(populations)”; the rationale for this was already described on lines 77-78.

Response: changed as suggested (current version: line 276, and lines 78-79)

On line 302, remove “) mm” following “6.4SD”.

Response: corrected typo (current version: line 307)

On line 405, delete “and use it” following “(Oyster Bay)”.

Response: changed as suggested (current version: line 414)

On line 457, delete the period following “J”.

Response: corrected typo (current version: line 473)

On line 511, change “M. Edie” to “Edie, M.”

Response: changed as suggested (current version: line 532)

Article titles for Aitken and Whitlock (2013), Camara et al. (2008) should be in sentence case. Kawecki and Ebert (2004), Palumbi et al. (1997) and one of the Whickham references (2011 or 2014) were not cited in the document.

Response: Titles corrected and un-cited references deleted from list.

Italicise *Ostrea lurida* in Trimble et al. (2009) and White et al. (2009).

Response: corrected as suggested

Remove the gazetteer names from the outplant sites in the caption to Figure 1. Suggested caption: Figure 1. Olympia oyster (*Ostrea lurida*) broodstock and outplant sites in Puget Sound, WA. Broodstock collected from Fidalgo Bay (F), Dabob Bay (D) and Oyster Bay (O). Outplanted to Northern (F), Hood Canal (D), Central (C) and Southern (O) sites.

Response: changed as suggested

Reviewer 2.

Line 38: I appreciate the use of cautious language “may“ but as you know resource managers and restoration groups have already done a lot of work with this species. The general reader would be better informed by a quick description (and citation) of the restoration activities that have already been undertaken for this species, especially in the Puget Sound.

Response: changed wording as suggested (current version: line 39)

Line 52: I apologize for the self-reference but Carson 2010 presents *O. lurida* data showing two peaks in settlement data (with a concurrent constant rate of brooding percentage). Those data seem very relevant to this discussion.

Response: changed to include findings by Carson 2010; added to reference list. Sentence now reads: “Carson (2010) likewise observed two settlement peaks (June and August) at two locales in southern California. In contrast, Seale & Zacherl (2009) observed only a single settlement peak (June) at two other southern California locales.” (current version: line 52)

Line 89: On behalf of my geologic colleagues I suggest calling Hood Canal a “fjord-like arm” or something similar. Apparently it is neither a fjord geographically (carved mostly out of unconsolidated material, not bedrock) nor geologically (the mechanism of carving was meltwater, not ice, making it a “tunnel valley”).

Response: changed as suggested. Phrase now reads ‘Dabob Bay is located within Hood Canal, a distinctly separate fjord –like arm of Puget Sound.’ (current version: line 90)

Line 105: It would be useful to describe the Clam Bay site a little more, perhaps in the preceding paragraph. Is it a “control” site only because it is not any of the three source sites, or for some other reason? Would you expect it to have characteristics similar to any of the three source sites that would make it less of a control?

Response: changed as suggested. Section now reads ‘In August 2013, 480 juvenile oysters (5-10 mm) from each population were placed at source locations: Fidalgo (N 48.478252, W 122.574845), Oyster (N 47.131465, W 123.021450), and Dabob Bays (N 47.850948, W 122.805694). In addition, oysters from each population were placed at Clam Bay (N 47.572894, W 122.547425), a site in central Puget Sound with generally moderate environmental conditions relative to the source locations (Figure 1).’ (current version: line 104)

Lines 111-116: More justification is needed here to support this choice. Was this because you feared survival would be very low at all sites if the oysters overwintered in place? It seems like “natural” overwintering conditions might be a part of local adaptation so it would be useful to know the strategy here.

Response: changed as suggested. Phrase now reads ‘were subsequently suspended from floating structures to avoid the risk of mass mortality (not due to selection) by reducing exposure to extreme temperatures during tidal exchanges’ (current version: lines 114-119)

Line 211: A placeholder (XY%, ± YZ SD) was not filled in with numbers here.

Response: summary data included. (current version: line 215)

Lines 231-238: It would be useful to include some of the actual percentages brooding here instead of just who was higher where.

Response: summary data included, cited Figure 14. (current version: lines 233-240)

Line 274: It's unclear what is meant by "reproductively discrete". It doesn't seem like the data exist to judge degree of isolation, if that is what is meant. Define or remove.

Response: removed words (current version: lines 275-276)

Line 277: Typo "indicator OF local adaptation"

Response: corrected typo (current version: line 278)

Line 285: I think you mean that the site had temperatures outside of Baker's thermal tolerance range for the species, but as written it implies that Baker measured temperatures at this site and that that range was exceeded.

Response: reworded as suggested. Phrase now reads 'the site exhibited temperatures outside of the thermal tolerance range reported by Baker (1995)'; (current version: line 286-287)

Lines 282-299: This would be a good place to mention the experimental design on floating structures as opposed to "natural" oyster populations exposed to low temperatures at low tide, and how that might influence the results.

Response: sentence added: 'Olympia oysters in Puget Sound naturally occur in seeps, perched lagoons, and other habitats that buffer temperature extremes; the deployment of experimental units suspended from floating structures or in a perched lagoon was an attempt to afford similar protection to the outplanted oysters.' (current version: lines 293-296)

Lines 326-328: This statement needs a reference to either published literature or your own data/figures. It also should be expanded to make clear what you mean. Is the Chl-A increased overall in the south? Or does an increase come earlier?

Response: reworded as 'The observed spawn timing also may be correlated with differences in chlorophyll a content between the Northern and Southern sites; the relationship of *O. lurida* spawn timing with primary production warrants further investigation.' (current version: lines 331-334)

Lines 350-352: Can you cite something here, perhaps from Mr. Blake's data or reports, regarding the distribution of oyster drills in Puget Sound?

Response: included a citation from Mr. Blake as suggested. Section now reads: 'Predation was also a factor in population specific survival at the Hood Canal site, where the Fidalgo Bay population had significantly greater mortality attributed to oyster drills. Fidalgo Bay has essentially no drills (B. Blake, unpublished data) to prey upon local oyster populations, whereas populations from Dabob and Oyster Bays may be adapted to environments with oyster drills.' (current version: lines 356-359)

Line 352: The shell thickness hypothesis seems like it would have been relatively easy to test after your experiment was concluded...

Response: in hindsight we agree with the reviewer, unfortunately lack of research funds at the time precluded this avenue of inquiry. (current version: line 360)

Line 391-398: In lines 67-70 you introduce two alternative hypotheses regarding false positives for local adaptation. I think it would be a good idea to make a clearer link between your language in this paragraph and those hypotheses.

Response: changed wording—section now reads, 'A reciprocal transplant using larvae coupled with molecular screening would be necessary to test the balanced polymorphism hypothesis. The differences observed could also be the result of low effective numbers of breeders in the hatchery, thus indicating a significant family effect and/or inbreeding depression as described in aquaculture of *C. gigas* (Camara *et al.*, 2008) and discussed as a potential issue for *O. lurida* restoration by Camara & Vadopalas (2009). Future genotyping and parentage analysis will shed light on the question of genetic drift.' (current version: lines 401-407 and lines 68-71 respectively)

Lines 417-524: This would be a good place to make some recommendations to restoration practitioners. Given your 3 concerns, what experiments or genetic tests might organizations ideally do before selecting broodstock or transplanting animals?

Response: added text: Given that population structure was detected among the only three locales examined in this study, more structure might be uncovered upon further investigation. Resource managers and restoration practitioners can minimize disruption of extant population structure, and increase the likelihood of restoration success by restricting transfers of stocks among locales; reliance on habitat restoration and natural recruitment is the most risk-averse strategy. To better understand risks to wild populations, biomarkers of adaptive significance are critical to pursue.' (current version: lines 434-440)