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Interactive comment on “The influence of food supply on the response of Olympia oyster larvae to ocean acidification” by A. Hettinger et al.

Anonymous Referee #1

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The influence of food supply on the response of Olympia oyster larvae to ocean acidification

A. Hettinger, E. Sanford, T. M. Hill, J. D. Hosfelt, A. D. Russell, and B. Gaylord

This is a very nice contribution by Hettinger et al that examines the implications of variable food and CO₂ levels for the performance of Olympia Oysters. They performed an experiment that examined three response variables and documented differential, significant effects on each variable measured. The experiments were performed well from a biological and chemical perspective. The results were clear and important. There are multiple good opportunities to improve this manuscript. I specifically believe some of the methods and statistical design require significant clarification. There are a series of key references what were not included. The discussion seemed too abbreviated as

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multiple opportunities to place the current findings in the proper perspective of current and former OA research were missed. This will be a good contribute to OA research.

Introduction and discussion: Add Gazaeu et al 2013 Marine Biology (has DOI number, not volume and pages, yet) as an excellent reference regarding general OA effects on bivalves.

Intro page 3, line 6: salinity, dissolved oxygen, and patterns of primary production. There are good references for these what should be cited.

Intro page 3, line 21: While Reisbell showed C:N ratios change, he did not examine larvae. Can you supply a references demonstrating that the C:N in Reisbell's paper are detrimental to the larvae?

Intro page 4, line 8: Holcomb et al., 2010, 2012; Melzner et al., 2011; Thomsen et al., 2013; Comeau et al., 2013. Talmage and Gobler 2012, MEPS, considered OA and altered food, specifically a harmful alga which is known to be a poor nutritional food source and could be considered here.

End of intro, page 5: "In these laboratory experiments, we paid particular attention to the possibility that negative effects of high pCO₂ might be ameliorated under high food availability." Its seems like this should specifically references the findings of Melzner et al 2011 and Thomsen et al 2013 or that the findings of Melzner et al 2011 and Thomsen et al 2013 should be explained in some detail somewhere in the introduction which sets up this hypothesis.

Methods, page 5, line 18: Given the subject of the paper, it was surprising the level of T-Iso fed the adults was not mentioned; please provide.

Methods, page 5, line 22: Comment on and provide a reference to put the larval density used (1000 per 4.5L) compares to densities in the field. This is very important, as the larval density is proportional to the nutritional demand and thus this will allow your food supply rate to be placed in its proper environmental perspective.

BGD

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Methods, page 6, line 6. $1000 \mu\text{atm}$ is also the level found in upwelled water on the US west coast (Feely et al 2008, Science). I believe this is worth pointing out perhaps while pointing out the finding of Barton et al 2012 that modern day CO_2 -enriched seawater can impact bivalve larvae.

Methods, page 6, lines 22-24. Provide the mean and the range of food levels found. As stated, the value provide ('can reach 24.6 mg/m^3 ') makes it sound like a maximum. True? This would be very high. Stating the mean and range would be of greater value to the readers.

Page 7 lines 12-17: "These buffers were made in-house and checked against a certified TRIS buffer (A. Dickson, Scripps Institute of Oceanography, La Jolla, California)." And how did they check out? Please provide a percent similarity \pm SD. Similarly, provide a level of accuracy attained via measuring Dickson's TA standard. Similarly, it would give readers confidence if they had a sense of how the measured pCO_2 levels compared with those calculated using CO_2SYS using TA and pH.

Page 8 lines 1-9. Drying day old larvae would seem to alter their size (shrink). Please comment on how drying for 24h effected the larval size in the methods.

Page 8, line 15: The use of AFDW on calcifying organisms can present an issue as shell can volatilize resulting in bias of the results, see (Gouletquer and Wolowicz, 1989) and salt residue on un-rinsed larvae may also contribute to measured weights (Moreno et al, 2001). Can the authors demonstrate that is not a concern in this case?

Page 8, line 20: Very clever use of the plates! I think it would be very useful to state precisely how you interpret settlers on plates. Is the assumption that those that do not settle have perished? Do any individuals settle on regions besides the plates? Is that possible?

2.5 Statistical analyses: The design here was unexpected as its not clear why headspace was a treatment factor that was considered statistically. It was not clear

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how the headspaces would be different, even upon re-reading the methods several times. I think the headspaces required further clarification earlier in the methods and an explanation here as to why it was evaluated statistically.

Also, can the authors defend the use of their nested ANOVA multiple times (multiple days) as opposed to using a repeated measures ANOVA for the course of the experiment?

Results: Again, the headspaces does not make sense. Why would the flasks differ based on this? If this is because the details are in the author's 2012 paper, they should make the effort to add those details to the methods here, as presently, why headspaces would be different or test is not clear

Between the pH rising and the alkalinity not changing (= no uptake of nitrate or phosphate by algae), this suggests the cultures were in stationary phase growth which, in and of itself, alters the nutritional content of the algae. As such, a greater effort should be made in the methods to describe the growth phase of the algal culture (exponential vs stationary phase growth). To be clear, this is not nit-picking but rather plays directly to the entire theme of the paper: Algal food quality changes as a function of the growth phase and condition of the algal cells. For example, the C:N ratio change through the growth cycle can be larger than the C:N ratio the authors referenced in the introduction in the Riesebell et al 2007 paper.

Please describe the number of settlers in terms of percentage of total in the figure and/or results.

Discussion: Overall, the discussion was surprisingly brief. To the point is good, but some good opportunities were missed here. The authors should consider some of the following points. 1. The effects of CO₂ on growth here were tiny (10%) compared to the very large effects on settlement (70%). Why would this be? What are the implications for early life history oysters?

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2. Upwelling of nutrients enhances primary production and thus counteracts acidification effects. This was discussed, but I think the paper would benefit from some further detail on this. How long to the low vs high pH / CO₂ periods last during upwelling events? How does the timing and duration of upwelling compare to the larval stages for Olympia oysters in this region? 3. How do the precise physiological effects of less than ideal food described here compare to the findings of Holcomb et al., 2010, 2012; Melzner et al., 2011; Talmage and Gobler 2012, Thomsen et al., 2013? Given this number of studies, can we now draw some larger conclusions about food level and CO₂ or are we getting different answers for different organisms? The experimental design and results here are quite similar to Melzner et al., 2011 and Thomsen et al., 2013, so I believe expanding on the parallels there is warranted.

Page 12, line 9: “in the absence of high seawater pCO₂” . Given you talk about high CO₂ water next, it would seem better to use the term low CO₂ here instead of “in the absence of high seawater pCO₂”

Page 12: Seems appropriate to make the linkage to the upwelling / oyster larvae hatchery paper by Barton et al 2012 in this portion of the discussion.

Page 13: “Our results suggest that Olympia oyster larvae do not demonstrate the ability to counteract exposure to elevated pCO₂ conditions in high food environments.” This statement is not reflective of the data in the paper. Better to state “Our results suggest that Olympia oyster larvae do not demonstrate the ability to fully counteract exposure to elevated pCO₂ conditions in high food environments

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