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

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Author response in bold italics:

We would like to thank Anonymous Reviewer 2 for his/her review of our manuscript. The comments, questions, and suggestions raised by the Reviewer have improved our manuscript. Below are our point by point responses (in bold italics) to all issues raised by Reviewer 2. The manuscript has been revised accordingly.

Anonymous Referee #2
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Experimental design and Analysis: The description of the experimental design could be clearer, especially whether larvae differed among jars. Jars could be and should be factored into the analysis, so that a 3 way rather than a 2 way ANOVA is done. If following this there are no differences due the jars, then this can be pooled following established procedures described in Underwood 1997 – for a more powerful test of hypotheses. This confusion arises because of somewhat contradictory statements. For example: Page 5786 Line 21 states that the design was 2 pCO_2 levels, 3 food levels and there were 5 replicate jars. In this case jars are replicates and not a source of variation On Page 5788 it states that headspaces were nested within pCO_2 level, which provides support for an argument that a determination should be made about whether differences exist at the level of jars – jars being nested in pCO_2 . It appears that a 3 way ANOVA is possible for some variables i.e. n=10 larvae were removed per jar.

We used Dixon's test for outliers to determine if there was any one jar within each pH level that contained samples outside of the normal distribution. We did not detect any outliers, which suggests that responses were consistent among replicate jars. The measurements we took on each individual larva are subsamples, and to include them in the analysis as co-equal measurements would be a case of pseudoreplication. To avoid this in our design, we consider the jars to be our sampling unit, and thus analyze mean responses from each jar using ANOVA. Because the jars were nested within the headspaces, we were interested in whether there was a significant effect of the headspace. For this reason, we include Headspace[pCO_2] as a factor in our analyses. These results are now presented in supplementary statistics tables. Our experimental set-up has also been clarified in the methods.

Description of Results: The description of results throughout pages 5789-5791 is frequently interrupted by the results of ANOVAs. These results need to be put in a table which can then be referred and in part this will solve the disjointed nature of the sentence structure and ensure better flow. Once the ANOVA tables are sorted out, there also needs to be an improvement in the description of the results in general. Also in this section there are several lines 12-18 dedicated to non-significant differences in interaction effects, although good this makes the text disjointed. If

this is ALL put in a table then the text will be much clearer and reading the manuscript significantly easier. In the time pressed world of science this will be valued.

We moved many of our ANOVA results to supplementary tables, and now emphasize only the statistics that are related to our central questions. We have also worked to present our results more clearly.

In section 3.2 page 5790 it states that there was an effect of food level at days 5,9, and 11 and then on day 11 there was also an effect of pCO_2 level. On referral to the figure, the difference in food is indicated by letters (A or B) above the columns, but there is no difference in the letters above high and low CO2 level at day 11 in the low food treatment. The text contradicts this statement, saying that there is ANOVA $P < 0.0471$ line 10. The question then for the reader is whether Tukey HSD post hoc test did not detect a difference between means, or whether the letters only indicate differences in means among food levels. This is very confusing for the reader and requires clarification.

The Tukey HSD was for Food Level, and not for pCO_2 and thus the shared letters above the bars in Figure 1 indicate food levels that did not differ significantly. This has been clarified in the figure caption.

Section 3.3: There are similar issues as described above with section 3.3, but here there are significant interactions, especially on day 5. This should be explained first because they are the level of significance which is more important and override the significance of the main effects. The significant differences among means are still not clear on Figure 2.

Again, the Tukey HSD was for Food Level, and not for pCO_2 and thus the shared letters above the bars in Figure 2 indicate food levels that did not differ significantly. This has been clarified in the figure caption. As for the significant interaction on day 5 for total dry weight, we have discussed this result (Pg 13, line 20-21). This was likely driven by the high total dry weights estimated in the ambient pCO_2/medium food level treatment (see Figure 2, panel A, the 2nd black bar).

Introduction and Discussion: Overall this was a very well written manuscript providing data answering a key question on the role of food in ameliorating at high but not low food concentrations the effects of pCO_2 .

Page 5782, lines 19-25: reposition and integrate into page 5783 line 15. Much stronger opening if you start with the first paragraph on page 5783.

We have reorganized the beginning of the introduction as advised.

Page 5793. Lines 15-17: Further commentary here on the food concentrations used in other studies may be beneficial. For example, in many lab studies it is standard practice to use high food abundance to ensure maximum survival of larvae - and these studies have still found a difference. Also in studies such as Dupont et al. 2010, working on lecithotrophic larvae with endogenous food supply, there was increased growth rate and no visible effect of elevated pCO_2

on survival or skeletogenesis. Some further commentary on endogenous and exogenous food supply would be useful.

The mode of larval development (i.e., planktotrophic vs. lecithotrophic) could certainly play a role in larval responses to stress, and responses will not only depend on innate physiological differences in species with different modes of development, but also on how food resources (e.g., phytoplankton) are expected to change. Changes in exogenous food sources could have impacts for larvae using both modes of development: directly on feeding, planktotrophic larvae, and indirectly on lecithotrophic larvae if adults (i.e., mothers) food supply changes and with it, her subsequent resource partitioning to eggs. We have added commentary of such factors to the discussion.