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Interactive comment on "Ocean acidification challenges copepod reproductive plasticity" *by* A. Vehmaa et al.

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We greatly appreciate the constructive suggestions on our manuscript. Please find below our detailed response to the comments, point by point, for our manuscript "Ocean acidification challenges copepod reproductive plasticity" by Vehmaa et al.

Anonymous Referee #1 Received and published: 19 January 2016 General comments: This work explores how wild copepods respond to varying ocean acidification scenarios during a large scale mesocosm experiment as well as examining the presence of possible maternal effects. The researchers have examined maternal effects using an egg transplant experiment where eggs of females from acidified con- ditions were incubated under identical conditions or ambient conditions. This study is interesting and

novel in the sense that a classical mesocosm experiment is combined with a laboratory approach, thereby opening the possibility to examine potential ma- ternal effects. The experiments produced several interesting results, the main findings being that animals from mesocosms exposed to elevated pCO2 are generally smaller compared to those exposed to ambient conditions (p=0.040), although no effect of el- evated pCO2 on egg production (0.137), nor the hatching success for the spawned eggs (p=0.052), was be observed. Further, the egg transplant experiment also shows that eggs produced at elevated pCO2 generally performed worse when incubated un- der similar elevated pCO2 conditions, than when incubated at ambient condition, with regards to hatching success (0.043) and nauplii development (p=0.047). The authors have used the appropriate statistical methods to analyze the results and generally present a nice discussion where they put their finding in the context of findings from other relevant studies. I generally find the manuscript to be well written, thorough, and easy to read and understand. However, there are some issues that should be resolved before this paper is ready for final publication. My main concern is that the authors report hatching success to be negatively affected in the mesocosm experiment despite a p-value of 0.052. I also think that the authors should consider the strength of the effects (how much is the different parameters affected (i.e. % change vs. control)), and not only rely on significant differences, when they discuss and conclude on the sensitivity of the investigated species to ocean acidification conditions.

Author response: Thank you for the constructive comments. It is obvious that we have not clearly differentiated between mesocosm hatching results and the egg transplant hatching results. The significance level used was 0.05 throughout the manuscript, indicating that p=0.052 is not statistically significant. We will clarify this in the revised manuscript. We will also pay more attention to the strength of the effects.

Specific comments: P18541: The title does not fully cover the findings in this study, since it gives the impression that only reproductive plasticity is examined. For instance, the authors found evidence that the female size is reduced by elevated pCO2 in the

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mesocosm experiments. I suggest changing “reproductive plasticity” to “phenotypic plasticity”.

Author response: We will follow the suggestion and change the title.

P 18544, line 3-7: The authors should also mention the studies that have reported negative effects of elevated pCO2 at levels relevant for year 2100 (e.g. Fitzer et al. 2012).

Author response: We agree with the reviewer. It is important to mention also studies reporting negative impacts. We will add a sentence on this.

Page 18545, line 1-: The authors present hypothesizes for the egg transfer experiment, but no hypothesizes are presented for the mesocosm experiment. Why not?

Author response: Thank you for this comment. We will add hypotheses for the mesocosm experiment part.

P18550, line 10: By writing “even though they differed between the mesocosm” readers might be lead to believe that there were in fact statistical differences. I recommend that the authors try to reformulate this or remove this part of the sentence.

Author response: We will reformulate the sentence.

Page 18550, line 21: In the results the authors write: ”Both fCO2 and TPC (<55 μm) had significant negative effects on EH (Table 4).” And in page 18552 line 5-7 they state: “Nevertheless, we found significant negative effect of ocean acidification on egg hatching success and adult female size”. However, the generalized linear mixed model for egg hatching success presented in table four list that pCO2 displayed a p-value of 0.052. I find this confusing! The authors make use of hypothesis testing throughout the MS but do not state the level of significance in the section regarding statistics under M&M. The principles for hypothesis testing state very clear the null hypothesis cannot be rejected when the significance level observed in a test is larger than the chosen significance level. In this case there is no evidence that the tested parame-

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ter has any significant effect (I am also very skeptical to formulations such as near significant/ borderline significant for that matter since the level of significance is absolute). If it is correct that the chosen significance level in the statistical tests is set to 0.05, the authors should refrain from referring to this result as significant throughout the manuscript, and instead threat it as not significant. I would also like the authors to state explicit the chosen level of significance in the M&M section.

Author response: Thank you for pointing out this mistake! The final hatching success model, which included both fCO2 and TPC was the best model even though the p-value for fCO2 was not <0.05. We apologize for this. We will do the necessary changes to correct this mistake and avoid further confusion. The significance level was 0.05 throughout the manuscript, and we will add this information to the Materials and Methods section.

The effect of pCO2 on hatching was actually tested twice in this manuscript. When comparing the ratio of hatching success in eggs incubated in mesocosm vs. common garden conditions the authors did find a significant effect on egg hatching success (see table 5). The fact that the effect of pCO2 on egg hatching success was tested twice, and found to show conflicting results, makes it confusing for the reader to know which results the authors refer to. I propose that the authors try to state explicit throughout the paper which experiment they refer to when reporting on hatching success (i.e. abstract, results, discussion).

Author response: The reviewer is correct. Testing hatching twice can be misleading as shown already in the earlier comments. We will clarify this in the revised manuscript.

Page 18550, line 21: "Both fCO2 and TPC (<55 μm) had significant negative effects on EH (Table 4)." It would be interesting to include an investigation of the correlation between fCO2 and total particulate carbon. A high correlation between these two parameters could suggest that elevated pCO2 may have stimulated the primary production in the treatments.

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Author response: As stated in the Materials and Methods, collinearity between all explanatory variables was checked, and it was concluded that they can be used in the same models. Primary production was not stimulated by elevated CO2; however, respiration was higher in the ambient treatments (Spilling et al., 2016). For more information on the effect of CO2 on organic matter, please see also Paul et al. (2015) www.biogeosciences.net/12/6181/2015/.

P18552, line 25-30: The authors should mention the development delay observed in the cited study by Pedersen 2014a.

Author response: We will add more information of the observed development delays in the cited studies.

Page 18547, line 14-16: "All the Acartia sp. adults and nauplii were considered to be species A. bifilosa because the other Acartia species in the area, A. tonsa does not usually exist in the area in early June (Katajisto et al., 1998). I find this to be a big assumption. A lot of factors could have changed the phenology of these species during the 17 years that have passed since the observation of Katajisto et al. The authors should run genetic analyses on a representative selection of the animals to confirm which species they have investigated and to make sure that it was not in fact a mix of several different species. Alternatively, the authors should refrain from stating the species name and instead refer to the animals as Acartia sp. throughout the MS.

Author response: Even though we are confident that all the animals used in these studies were A. bifilosa, we cannot be 100% sure. We have therefore decided to change the species name to Acartia sp.

P 18550, line 11-13: "Prosome length (PL) of A. bilfonsa increased during the first week of the study, however there seemed to be differences between the mesocosms already at the start (Day 3, Fig. 1b)." Here, and other places in the MS where significant differences are reported, the authors should provide some information regarding the strength of the effect. How large was the percentage difference in size between

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the different exposure groups and the control? This type of information is especially important when trying to assess the ecological importance of observed effects. The authors should provide this kind of information in those cases where a significant effect on endpoints is observed. I would also like the authors to try to make use of these estimates of observed differences in their discussion and try to discuss their possible ecological implications.

Author response: We will pay more attention to the description of the results. Please notice that the first sampling day (Day 3) was not included in the PL analysis. The sentence refers to figure 1b, which shows the average prosome lengths of individuals, collected from the mesocosms each week. We will make sure that every time we mention statistical difference, we refer to the table presenting the test statistics.

P 18553, line 8-9: "This suggest that A. bilfonsa and its reproduction are after all fairly sensitive to ocean acidification." I think that this conclusion is stretching the result too far. If the species is "fairly sensitive" one would expect to see an effect on the investigated reproductive parameters (egg production and hatching success) in the mesocosm experiment. However, this experiment did not directly reveal any significant reduction in reproductive parameters, although a small reduction in size was observed among the females that developed under elevated pCO2 conditions. Only the transplant experiment was able to show a small negative effect of elevated pCO2 on hatching success and development index. I therefore think that the authors should tone down the language regarding the sensitively of their model species.

Author response: This sentence refers to weakening maternal provisioning during the experiment, and to the observation that maternal effects are weaker, not stronger as hypothesized, in high fCO2 conditions. We will tone down the sentence.

P18554, line 20-23: I find it speculative to draw conclusions based on a very modest difference in correlation coefficient and advice that this argument is removed. The authors are encouraged to provide statistical evidence showing that the lines differ.

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Author response: We will delete the sentence.

P 18555, line 16-20: “Since it takes 8.5 days for a sixth stage nauplius of A. bifilosa to develop through the five copepodite stages and reach adulthood at 17âŮęC (Yoon et al., 1998), it is plausible that at 9-11âŮęC the copepods could have also developed through several stages causing the differences in prosome length between the treatments on Day 10.” Using a temperature equation (e.g. a Belehradek-equation or similar) for the development rate in this species would make the argument more concise.

Author response: Thanks a lot for the suggestion! If Acartia sp. development follows the Bělehrádek’s temperature function, it would take 12-15 days for VI stage nauplii to reach adulthood at 9-11°C (Bělehrádek, 1935; McLaren, 1966). The constants used in the equation (α=1008, a=-8.701) were the same as in Dzierzbicka-Glowacka et al. (2009) for A. bifilosa. We will add this information to the manuscript.

P18556, line 1-2: This part of the sentence is confusing; “however, the expected effect would be positive”. How can food quantity or quality be “positive”? I suggest that the authors change the argument to apply to; ”increased food quantity and higher quality”.

Author response: The sentence needs clearly rephrasing. We will clarify this in the revised manuscript.

Table headings: I find the descriptions for table 1, 2 and 4 very short. It should be stated what “value” refers to. Please provide more information so that the tables become more self-explanatory.

Author response: We will rewrite the table headings and add more information to them.

Technical corrections: P 18543, line 11-14: The last part “could be fairly plastic..” does not go well together with the first part.

Author response: We will rewrite the sentence.

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P18550, line 11: The authors should note that the protosome length was measured on females.

Author response: We will add this information to the revised manuscript.

P 18553, line 23: I suggest that the authors change “overestimate” to “over- or underestimate”, as both of these can result from short-term results focusing on a limited number of life-stages.

Author response: We will make the suggested change to the revised manuscript.

P18554, line 7: I don’t understand why the authors write “however” in this sentence.

Author response: We will delete the word however.

P185566, line12-14: Please modify the sentence so that it makes better sense.

Author response: We will modify the sentence in the revised manuscript.

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