

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

### **Anonymous Referee #1**

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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 conditions 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 maternal effects. The experiments produced several interesting results, the main findings being that animals from mesocosms exposed to elevated pCO<sub>2</sub> are generally smaller compared to those exposed to ambient conditions ( $p=0.040$ ), although no effect of elevated pCO<sub>2</sub> on egg production (0.137), nor the hatching success for the spawned

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eggs ( $p=0.052$ ), was observed. Further, the egg transplant experiment also shows that eggs produced at elevated  $p\text{CO}_2$  generally performed worse when incubated under similar elevated  $p\text{CO}_2$  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.

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  $p\text{CO}_2$  in the mesocosm experiments. I suggest changing “reproductive plasticity” to “phenotypic plasticity”.

P 18544, line 3-7: The authors should also mention the studies that have reported negative effects of elevated  $p\text{CO}_2$  at levels relevant for year 2100 (e.g. Fitzner et al. 2012).

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

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.

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Page 18550, line 21: In the results the authors write: "Both fCO<sub>2</sub> 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 pCO<sub>2</sub> 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 parameter 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 treat it as not significant. I would also like the authors to state explicit the chosen level of significance in the M&M section.

The effect of pCO<sub>2</sub> 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 pCO<sub>2</sub> 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).

Page 18550, line 21: "Both fCO<sub>2</sub> and TPC (<55 μm) had significant negative effects on EH (Table 4)." It would be interesting to include an investigation of the correlation between fCO<sub>2</sub> and total particulate carbon. A high correlation between these two parameters could suggest that elevated pCO<sub>2</sub> may have stimulated the primary pro-

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duction in the treatments.

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

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.

P 18550, line 11-13: “Prosomal 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 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.

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

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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 pCO<sub>2</sub> conditions. Only the transplant experiment was able to show a small negative effect of elevated pCO<sub>2</sub> on hatching success and development index. I therefore think that the authors should tone down the language regarding the sensitivity of their model species.

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.

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 17°C (Yoon et al., 1998), it is plausible that at 9-11°C 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.

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”.

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.

Technical corrections: P 18543, line 11-14: The last part “could be fairly plastic..” does not go well together with the first part. P18550, line 11: The authors should note that the protosome length was measured on females. 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. P18554, line 7: I don’t understand why the authors write “however” in this sentence. P185566, line

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12-14: Please modify the sentence so that it makes better sense.

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**BGD**

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