

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

**Anonymous Referee #2**

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The manuscript investigates the effects of ocean acidification reproduction of a Baltic Sea copepod and a potential role of the production of antioxidants for a better quality of the offspring. The effects of changing pH on the performance of zooplankton are at present in focus of the scientific community, and a large number of publications – mostly laboratory studies on reproduction– have been published in recent years. Although focusing on a timely topic, the manuscript is seriously flawed. As can be seen from the many comments below, I have problems with the strong focus on adaptation/plasticity in the introduction/discussion for which barely relevant data is presented and some conclusions which are not supported by careful interpretation of data. Most relevant, however, is the relatively weak experimental quality of the study which is below the requirements of Biogeosciences. Replication is lacking in most experiments,

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egg hatching and development is based on a low number of observations. While the flawed interpretation of results might be corrected (see details below), the methodological issues cannot. Therefore, I the MS does not have the quality to be published in Biogeosciences.

Introduction:

p. 188543, line 4: I don't understand the context of plasticity and rapid change postulated here. Research has generally shown that oceanic copepods living in less variable environments have a large plasticity to pH beyond that of year 2100 scenarios (there are now several reviews available on this topic which should be cited; the few studies highlighted for negative effects in a later §are rather exceptions than the rule). This suggests that there might not be a significant selective pressure for a larger plasticity towards pH as suggested by the authors. In addition, I wonder why the focus is primarily on plasticity. The results presented here do not relate much to this or the underlying mechanisms (physiological, genetic). Finally, I miss the justification of the study in the seasonal context. In the seasonal variable environment the MCs were located, pH is driven up by the biological activity in spring, followed by the increase in production of heterotrophs. I therefore wonder about the pH conditions likely experienced by copepods in different climate change scenarios. Certainly, they will not experience equilibrium conditions. I miss a few words on this in the introduction. The only rapid and unusual change experienced by the species is the one associated with the very rapid decrease in pH at the beginning.

p. 188543, line 26: Bron et al. might not be the original source for the information provided here, as well as Beaugrand et al. 2003 certainly does not provide original evidence of the diets of several important fish species. Whether zooplankton control harmful blooms is also disputable, the lack of grazing is more often inferred as a reason for bloom formation. Again, original literature should be cited.

p. 188544, line 26: I don't understand how transferring the eggs from one mesocosms

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to the outside conditions contribute to the characterization of plasticity.

p. 188545, line 1: The first hypothesis needs more explanation; the preceding paragraphs do not provide evidence for this. And how is this related to the evaluation of plasticity in different environments postulated in these paragraphs? Little background is also provided for the second hypothesis. Material and methods:

p. 188545, line 9: More background knowledge is needed to understand the set-up. What pH had the water before the pH was adjusted to different levels? Timing of the experiment related to the seasonal phase of the system? Was the pH kept constant over the 45 days?

p. 188545, line 17: from day 24 to 45 sampling was not weekly.

p. 188546, line 10: Was the pH measured after the incubation? pH should increase due to low light conditions and heterotrophic activity. p. 188546, line 10: Why were no samples taken to count eggs already present in the incubation water? The procedures described to account for this in a later step are not convincing because egg development time at 10 degrees is likely longer than 24 hours.

p. 188546, line 14: The copepods are small, likely due to shrinkage in RNA later. This is critical as no information is available whether this affects all specimen in the same way (several preservatives due not). Anyways, females should have measured before preservation, as no biomass estimates are possible. EP should have been normalized to the strong variation in size, which is not possible anymore.

p. 188546, line 17: How many eggs were incubated per treatment? EP was very low during large parts of the experiment suggesting that only few eggs were incubated per treatment because of they needed to be divided between pH and outside MCs treatments. The number of eggs appears to be by far too low for reliable estimates of hatching.

p. 188546, line 19: I am not convinced that these are common garden conditions,

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as it is expected that outside conditions were closest to the low CO<sub>2</sub> treatments; in consequence, transfer stress is largest for eggs transferred from high CO<sub>2</sub> into outside conditions, which potentially bias the results. Parafilm is not airtight, consequently pH conditions were not constant during egg hatching incubation.

p. 188547, line 1: This procedure is not convincing as it assumes that hatching time is shorter than 24 hours. This is not the case.

p. 188547, line 10: The lack of replication is seriously critical especially in the development experiment, but also for EP and EH. In addition, estimates of EH are based on small numbers, as are those of 'development'. Considering the bias due to introduced eggs and nauplii with the incubation water, this is not state of the art and below the experimental quality required for a journal with high impact factor.

p. 188548, line 8: TPC is a poor predictor for feeding conditions of copepods, which feed generally on food larger than 10  $\mu\text{m}$ . Results:

p. 188550, line 6: Error bars are missing in all figures; methods should give more details on the number of eggs incubated for hatching. Is the increase from day 3 to 10 significant? When size varied, EP should be normalized. In Table 3 units are missing. The table needs explanation as it contains only limited information on the variation of environmental factors. What does 'since start' mean? A graph giving their temporal variation would be much more interesting. May be I am wrong, but must there not be 3 days averages for each time egg production was measured? What about changing food composition in terms of size and species composition. Food > 10  $\mu\text{m}$  is usually a much better predictor of egg production than < 55  $\mu\text{m}$ . How do the authors else explain the variation in egg production with the low variation observed in Chla? Acartia species are known for their omnivory, and heterotrophic food is not included in quantitative estimates of food abundance. This might very well influence and bias any statistical analyses.

p. 188550, line 11: Again, error bars are missing. A major reason for changing size

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is the maturation of new females. The size increase seems to be delayed in the MCs with lower pH and therefore Information on the pH prior to pH adjustment must be provided. Regarding EH the authors should be careful not to emphasize differences of a few percent, especially considering that no information about the number of eggs is provided and no replication was done.

p. 18551, line 4: I have some doubts whether these are common garden conditions. Based on the provided information on the set-up of the experiments (which is poor), one would expect from the natural seasonal variation of pH in the coastal Baltic that the common garden conditions are close to the lowest CO<sub>2</sub> treatment. These are not common garden conditions. Anyways, environmental conditions in the common garden must be presented.

p. 18551, line 14: Which adaptive maternal effects are meant here, and why adaptive? As outlined above females in the high CO<sub>2</sub> treatments were likely exposed to largest differences between start of pH lowering and first experiments. I would conclude that acclimation time to a drastic decline in pH was too short, but as soon as next G developed effects vanished. This has nothing to do with adaptation. Anyways, results should not be interpreted at this point.

Discussion:

p. 18552, line 2: T has a strong influence on the efficiency with which food is utilized by copepods, particularly, when food resources are limiting as in the MCs. Although T did not vary among the MCs, it increased over the first two weeks from 9 to 15 and, therefore, has an interactive influence on the efficiency of food utilization together with food conditions in each of the MCs. Thus, T needs to be included in the analysis. Why 'phenotypic buffering'?

p. 18552, line 6: I wonder how much of the significance of pH effects on hatching and size is influenced by day 3 measurements. The MC set-up introduced likely some strong, artificially rapid decrease in the pH in those MCs with very high CO<sub>2</sub> (the au-

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thors must report the initial pH before acidification) during the first days. This has to be taken into account when comparing responses of copepods. Any delay induced in the development of a cohort due to the rapid change (which took place because size increased in all mesocosms!) has therefore a strong influence on the interpretation of the results at particular days and needs to be taken into account; and cannot be interpreted as threshold. Again food conditions and T interact in influencing also size of females, making the analysis of the influence of T, food and pH difficult. After 10 days size and hatching (which was in all MC > 90%) was rather similar, pointing to no strong pH effects as claimed here. In this context, I would like additionally to emphasize the methodological limitations of the study that make interpretation also difficult (see above, e.g., the lacking replication, low number off eggs, inappropriate development experiments). Anyways, the artificial rate of change in the beginning needs to be taken into account. In my opinion, the conclusion of pH effects on size and hatching and lacking adaptive maternal effects is not supported by evidence.

p. 18552, line 9: Here I miss an evaluation whether food < 55  $\mu$ m actually can show what the authors wanted to show. This size choice is against many other studies that show for instance a much better predictive power of food estimates > 10-20  $\mu$ m. How is the increase in EP by a factor of 3 explained?

p. 18552, line 16: I find this confusing: Table 3 shows low concentrations and small ranges in TPC. This obviously contrasts the statement here that there was a sharp decline in Chl a.

p. 18552, line 19: Hatching of eggs was > 85% in the majority of the incubations over a variation in ORAC by a factor of three. In addition, there are many other factors influencing egg hatching success, particularly composition and quality of food. I am not convinced that ORAC in females is the main factor influencing egg hatching. The authors postulate a threshold around 800-1000  $\mu$ atm; still hatching was > 90%, and again the problem of lacking replication and estimates of variability exist in addition to the considerable low numbers of eggs that were used in experiments.

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p. 18552, line 22: Results on development should be shown, and details on the number of nauplii examined should be provided.

p. 18553, line 1: The authors are analyzing here differences in egg hatching of a few percent based on estimates that are seriously flawed by the experimental quality. I am not convinced.

p. 18553, line 22: The relevance of transgenerational effects for interpreting the present results needs explanation. In addition, what is the potential influence of changing T over time for the interpretation of the observation in comparison to other studies?

p. 18555, line 1: Again the interpretation of the cause of the effects on size suffers from adequate measurements of food quantity available to copepods. In addition, an evaluation of the variability in size is lacking, and the measurements are based on insufficiently low female numbers. There is also some variability in the estimates (e.g., MC6). Any suggestions? Moreover, the generalization to 'high' CO<sub>2</sub> is not supported by data, as at 1000 ppm, size doesn't seem to be influenced much. In addition, the problem exists that due to potential delays in development caused by an initial pH 'shock', the conditions for cohort development (food, T) differ among MCs. For instance, a delay in MC 8 might have caused a cohort of copepods to develop at suboptimal food conditions at a different T (as indicated by EP). Thus, results are not directly comparable with regard to pH.

p. 18557: Conclusions: The generalization from effects of mineral composition (C/N) to food quality is doubtful.

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Interactive comment on Biogeosciences Discuss., 12, 18541, 2015.