Interactive comment on “Ocean acidification challenges copepod reproductive plasticity” by A. 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 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 pCO2 are generally smaller compared to those exposed to ambient conditions (p=0.040), although no effect of elevated 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 mesocosm experiments. I suggest changing “reproductive plasticity” to “phenotypic plasticity”.
Author response: We have followed the suggestion and changed 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 have added a sentence on this. P 3, lines 23-26.

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 have added hypotheses for the mesocosm experiment part. P 4, lines 27-29.

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 have reformulated the sentence. P 10, line 1-2.

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 through- out 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 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 fCO_2 and TPC was the best model even though the p-value for fCO_2 was not <0.05. We apologize for this. We have done the necessary changes to correct this mistake and avoid further confusion. The significance level was 0.05 throughout the manuscript, and we have added this information to the Materials and methods section. P 9, lines 22-23.

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 have clarified 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.
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 CO_2; however, respiration was higher in the ambient treatments (Spilling et al., 2016). For more information on the effect of CO_2 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 have added more information of the observed development delays in the cited studies. P 12, lines 7-15.

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 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 have paid 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 have made 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 fCO_2 conditions. We have toned down the sentence. P 12, lines 22-24.

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.
Author response: We have deleted 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 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.
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 have added this information to the manuscript. P 14, lines 25-28.

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 have clarified this in the revised manuscript. P 15, lines 14-15.

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 have rewritten the table headings and added 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 have rewritten the sentence. P 2-3, lines 31-3.

P18550, line 11: The authors should note that the protosome length was measured on females.
Author response: We have added this information to the revised manuscript. P 10, line 3.

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 have made the suggested change to the revised manuscript. P 13, lines 4-5.

P18554, line 7: I don’t understand why the authors write “however” in this sentence.
Author response: We have deleted the word *however*.

P185566, line12-14: Please modify the sentence so that it makes better sense.
Author response: We have modified the sentence in the revised manuscript. P 15, lines 25-29.

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, 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.

Author response: We thank the reviewer for the comments. It is unfortunate that our focus on maternal effects as a possible reason for the high buffering capacity of copepods in the face of ocean acidification did not convince the reviewer. The methodology used in the egg transplant experiment is published (Vehmaa et al., 2012) and we have conducted several laboratory-based OA studies using *Acartia bifilosa* as a study object (Vehmaa et al., 2012; 2013; Engström-Öst et al., 2014). The current study offered us a great opportunity to test previous results in a more natural environment in mesocosms. Until now, we had observed that *A. bifilosa* egg production rate is not affected by pH decrease predicted for the next century, but egg development might be. The egg transplant experiment used in this study enabled the possibility to separate the effect of OA on the reproducing female and on the developing egg. At least in this species, it seems to make a difference for the offspring development whether the eggs are laid in the same environment where they are developing (i.e., transgenerational plasticity). Also, when testing survival and reproductive success of a contemporary population in the ”future conditions”, we are testing plasticity of the animals.

The experimental set-up of the whole mesocosm campaign was planned in such a manner that there was an array of fCO_2 treatments that were sampled repeatedly. The set-up for this copepod experiment followed the overall sampling schedule. This was not an ANOVA-type of study, so the criticism of the lack of replication is not valid. Our studies were replicated in time and were analysed statistically using appropriate nested-designs.

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 plastic- ity 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.

Author response: We have a small dilemma here since the first reviewer recommends us to add references showing negative effects of OA on copepods, and the second reviewer thinks that the studies showing negative effects are rather exceptions than the rule.

Unfortunately, the reviewer did not mention any of the reviews that should be cited. Despite a thorough literature search, we are not sure which reviews should be added. The claim that research has generally shown that oceanic copepods living in less variable environments have a large plasticity to pH beyond that of the year 2100 is new to us, because our impression on the matter is the opposite. For example, reviews by Whiteley (2011) and Halsband & Kurihara (2013) state that species that are currently inhabiting fluctuating environments are likely to be more tolerant to ocean acidification than those adapted to stable conditions.

According to our understanding, plasticity is the ability of an individual or a population to alter its physiological state, appearance or behaviour in response to the environment (West-Eberhard, 2003). In the current study, one population of copepods was divided into mesocosms subjected to different CO_2 treatments. We followed copepods for several weeks and measured their physiological state (reproduction and antioxidant balance) and appearance (prosome length).

With respect to the seasonal context, the study was intended to cover the late spring/early summer period. For reasons outlined in the overview paper by Paul et al. (2015), this season was chosen to focus on the low productivity, i.e., the post-spring bloom period. Because of the low productivity during this time the pCO_2 in the enclosed and surrounding waters were comparatively stable over time (see Paul et al., 2015 for detailed information on the carbonate chemistry in the mesocosms and the surrounding waters). Over the annual cycle, pCO_2 and pH vary substantially at the study site as a result of biological activity and mixing/upwelling of CO_2-enriched deep water. There are also strong spatial gradients in seawater pCO_2/pH, most prominently between the surface layer and the CO_2-rich deeper waters. Thus, the copepods in the study area are likely to experience strong changes in seawater carbonate chemistry, both seasonally and during their diurnal migration. As outlined above, one might therefore expect the plankton community in the study area to exhibit comparatively high plasticity and low sensitivity. As requested by the reviewer, we have added some information on this issue in the introduction. P 4, lines 9-18.

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.
Author response: We have done our best to find original citations. P 3, lines 14-17.

p. 188544, line 26: I don't understand how transferring the eggs from one mesocosms to the outside conditions contribute to the characterization of plasticity.
Author response: With transgenerational plasticity we mean maternal and paternal effects. We have clarified the sentence. P 4, lines 21-24.

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.
Author response: We have aimed to clarify the origin of these hypotheses even more in the revised manuscript. P 4-5, lines 27-4.

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?
Author response: All mesocosms had a similar pH of around 8.0 before the pH adjustments using CO_2 saturated seawater additions. A second addition of CO_2 was made on Day 15 in the upper 7 m to counteract pronounced outgassing. Otherwise pH was allowed to fluctuate naturally.

We have added some more information (P 5, lines 9-20); however, more detailed information on the set-up, adjustment of the pH levels, as well as stability of pH over the whole study period can be found in overview paper by Paul et al. (2015). www.biogeosciences.net/12/6181/2015/.

p. 188545, line 17: from day 24 to 45 sampling was not weekly.
Author response: We have rephrased the sentence. P 5, lines 23-25.

p. 188546, line 10: Was the pH measured after the incubation? pH should increase due to low light conditions and heterotrophic activity.
Author response: pH was measured before and after the bottle incubations. We have added a table presenting these measured values as supplementary material (Table S1).

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.
Author response: We did not count the eggs already present in the incubation water, because there were no suitable methods for this. Eggs are not evenly distributed in the 55 m^3 mesocosms, so counting the eggs from an extra litre of water would unfortunately not have given us accurate information on the number of eggs in the incubation water. Handling of the incubation water, on the other hand, was restricted in order to keep the

natural plankton community and fCO_2 conditions as stable as possible. Please see also the responses below.

We do not consider the extra eggs in the incubation water to have caused a notable error in our results because the adult copepods perform diel vertical migrations, and stay below the surface layers during the time of our sampling (8:00-12:00) (Almén et al., 2014). Also, *Acartia* eggs sink (Katajisto et al., 1998; Katajisto, 2003), so the water sampler that took the integrated samples from 17 m to the surface would not have caught a large proportion of eggs laid during the sampling or some hours before.

p. 188546, line 14: The copepods are small, likely due to shrinkage in RNAlater. This is critical as no information is available whether this is 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.
Author response: The referee is correct that RNAlater can affect the size measurements (e.g. Foley et al., 2010). The effect depends on the number of segments in the animal, i.e., the more segments the larger effect. Shrinkage is approximately 15% for copepods (Prof. Elena Gorokhova, Stockholm University, personal communication). As all the measured copepods were adult females, we assume the shrinkage to be in proportion similar for all individuals, which means that our results are quite conservative, and the size differences could actually have been larger before preservation. P 6, lines 18-24.

Please notice that there was a typo in the title of y-axes in Fig. 1b. Naturally, it should be *Prosome length (mm)*.

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.
Author response: The median number of eggs incubated per petri dish was 49, and varied between 11 and 158. We agree that 11 eggs might not be an ideal number to make reliable estimations of hatching success. Therefore we are pleased that only on the Day 24 in the MC 7 both of the hatching conditions (MC and common garden) included less than 20 eggs. We have added a supplementary table showing the number of incubated eggs (Table S2). Further, a low number of eggs is not such a big problem when using GLMM with binomial error structure for data analysis. Even though the hatching results are presented as percentages, we did not use the percentage data in the model. Instead, we had two columns with unhatched eggs in one and hatched nauplii in the other. R accounts for sample size and the logit link function to ensure linearity (Crawley, 2009). We have confirmed this with the departmental statistician (Åbo Akademi University).

p. 188546, line 19: I am not convinced that these are common garden conditions, as it is expected that outside conditions were closest to the low CO2 treatments; in consequence, transfer stress is largest for eggs transferred from high CO2 into outside conditions, which potentially bias the results. Parafilm is not airtight, consequently pH conditions were not constant during egg hatching incubation.
Author response: The difference between pH treatments was maintained in the Parafilm sealed petri dishes during the hatching/nauplii development incubations. We have added

a supplementary table of the pH measured from the petri dishes before and after the hatching incubation (Table S1). This will also allow the readers to notify the fluctuating condition in the common garden treatments.

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.
Author response: Not to filter the water even though we might get carry-over individuals or eggs with the water was a compromise we had to do. Filtering of water affects the gas balance (Riebesell et al., 2010) and filtering would have thus affected the fCO_2 conditions of the water. We decided that we cannot risk the treatment condition in favour of no extra individuals or eggs in the incubations. We managed to figure out ways how to deal with these possible source of errors as described in the text. However, the reviewer is right that if extra eggs ended up in the egg production incubation bottles and if they did not hatch during the incubation, we could not separate them from the ones that were actually produced in the bottles. Anyway as mentioned already above, during the sampling the copepods are migrated deep and eggs broadcasted then are also deeper down in the water column, whereas eggs that are broadcasted in the surface layers at night have had hours to sink before sampling takes place (Holliland et al., 2012; Almén et al., 2014). In that sense, sampling schedule was nearly optimal for our purpose and sampling occurred when egg number was probably at its lowest in the water column on a 24 h schedule.

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.
Author response: The set-up was built so that we had replication in time and it was analysed as such. That means that the gained results are reliable. We strongly disagree with the reviewer that state-of-the-art studies should always be replicated, ANOVA-kind of set ups. Also, as noted already above, modern statistical analysis, such as GLMM, can take into account the varying sample sizes. Of course it would be great to have many replicates but there are limiting factors concerning how many replicates are possible to handle. In this case, also the number of animals that can be caught weekly was limited as the populations had to remain abundant in the closed mesocosms until the end of the seven weeks long experiment.

p. 188548, line 8: TPC is a poor predictor for feeding conditions of copepods, which feed generally on food larger than 10 µm.
Author response: Food larger than 10 µm is included in the TPC fraction of <55 µm.

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 µm is usually a much better predictor of egg production than < 55 µm. 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.
Author response: Please notice that the average values in Figure 1 are averages per bottle i.e., a) the total number of eggs produced divided by the number of females (~17) per bottle per day, b) average prosome length of ~15 females, c) hatching success (%) calculated using all the eggs on the petri dish, d) measurement based on a sample of ~30 females. Therefore no standard deviation can be applied here.

Our linear mixed model (LMM) did not test the difference between days. However, we tested the difference in EPR between Day 3 and Day 10 separately, and can therefore conclude that the difference is statistically significant (paired student's t-test: $t = -5.115$, $df = 5$, $p = 0.0037$).

There are also studies stating that in *A. bifilosa* female size and egg production rate do not necessarily co-vary (Koski and Kuosa, 1999). Therefore, we wanted to present size and egg production results separately.

We will add more information to Table 3. Unfortunately we cannot add a graph showing the variation in fCO_2, TPC (<55 µm) and C:N (<55 µm) during the study because those results are presented in Paul et al. (2015) www.biogeosciences.net/12/6181/2015/.

Please notice that total particulate matter larger than 10 µm is included in the fraction smaller than 55 µm. We are aware of that the fraction chosen to indicate the quantity of food is not perfect but it is the best one available. The other options would have been chlorophyll *a*, TPC (<10 µm) or biomass' of different phytoplankton taxa. As mentioned also by the reviewer, *Acartia* is omnivorous, and chl *a* or a selection of separate phytoplankton taxa would not describe its possible diet in a satisfactory manner. The used TPC fraction includes all particles that are smaller than 55 µm and contain carbon.

p. 188550, line 11: Again, error bars are missing. A major reason for changing size 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.
Author response: P 5, lines 13-14. fCO_2 in the mesocosms prior to the pH adjustments was 237±9 µatm and pH ~8. We have added this information to the manuscript. Please note that Day 3 was not included in the prosome length analysis.

As already mentioned above, we had replication in time, and the median number of eggs incubated per petri dish was 49. We will pay more attention to the strength of the effects; however, if the differences are statistically significant it is our duty to report them as such.

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 CO2 treatment. These are not common garden conditions. Anyways, environmental conditions in the common garden must be presented.

Author response: The common garden conditions are common conditions for the animals originating from the mesocosms at a certain time point. The conditions outside the mesocosms fluctuated more than inside them during the study, but they were anyway the same each week for all treatments. The analysis takes this into account by comparing hatching and nauplii development measured at the same time point. Nevertheless, we agree that using the name common garden can be misleading and we have changed it to Baltic water. We have added a supplementary table presenting the measured pH values before and after the incubation and including also pH values of the Baltic water (Table S1).

p. 18551, line 14: Which adaptive maternal effects are meant here, and why adaptive? As outlined above females in the high CO2 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.
Author response: We expected eggs to have higher hatching success and develop more rapidly in the mesocosm water compared to common garden (Baltic Sea) water because mothers are able to adjust their eggs to prevailing conditions (maternal effect). Moreover, the effect is adaptive because it increases the fitness of the offspring. Nevertheless, we will delete this sentence and interpret the results in the Discussion.

72-hour acclimatisation time have been used for CO_2-treatments even higher than the high treatments in this study (Cripps et al., 2014a; 2014b). Also, based on our previous experience with *Acartia* sp., three days is enough for this species to acclimatise to changed CO_2-conditions (Vehmaa et al., 2012).

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'?
Author response: Please notice that we have explained what we mean by phenotypic buffering in the Introduction, and also in the sentence in question in the Discussion. We agree that temperature influences copepods a great deal, and analysing temperature-food interactions would certainly be highly interesting. However, with a restricted data set, only the most interesting and justifiable variables should be tested to avoid over-parameterisation of the model (Babyak, 2004).

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 CO2 (the authors 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.

Author response: Please notice that Day 3 was not included in the prosome length analysis (Table 1). The significant negative effects of fCO_2 and TPC on copepod size were gained without data from that day. For egg production and hatching success, three days acclimatisation period was considered to be sufficient (e.g., Cripps et al., 2014a; 2014b; Vehmaa et al., 2012).

Again, we agree that analysing temperature-food interactions would be highly interesting, however impossible in order to avoid over-parametrization of the model. Hopefully we are able to test these effects in a future study.

We consider this to be a very successful study because of the high egg hatching success in all treatments. This indicates that the copepods were doing fine, and that the differences between the treatments were actually because of the treatment conditions, and not due to stressful lab conditions, bottle effects, or bad food etc.

Please see also the replies above concerning replication and the number of eggs in the egg hatching/development incubations.

p. 18552, line 9: Here I miss an evaluation whether food < 55 µm 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 µm. How is the increase in EP by a factor of 3 explained?

Author response: Please notice that the fraction of particulate carbon larger than 10-20 µm is included in the used fraction of smaller than 55 µm. As already mentioned above, TPC (<55 µm) was the best available estimate of copepod food quantity.

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.

Author response: Unfortunately, Table 3 shows only the range over the whole experiment, and the decline in primary production after Day 17 is not therefore visible in the table. Also, TPC and chlorophyll *a* do not necessarily co-vary since organic matter of heterotrophic origin is also included in the TPC analyses. For more detailed results of e.g., Chl *a*, TPC and fCO_2, please check Paul et al. (2015). www.biogeosciences.net/12/6181/2015/

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 µatm; 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.
Author response: Please notice that we are not at any point suggesting that ORAC in females would be the main factor influencing egg hatching success. We did test the effect of food quantity and quality on egg hatching success, and ORAC was not included in that generalized linear mixed model (GLMM). The measurements of female copepod antioxidant balance were done in order to provide possible additional information of the maternal provisioning on the offspring. We do not have proof that this would be a case of cause and effect, and that is exactly why we tested their correlation. We have clarified this even more in the revised manuscript. P 13, lines 21-22.

p. 18552, line 22: Results on development should be shown, and details on the number of nauplii examined should be provided.
Author response: We have added a supplementary table showing the total number of incubated eggs, as well as the number of hatched nauplii (Table S2).

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.
Author response: We consider it to be our duty to report statistically significant results and trust on them. As already mentioned several times above, we do not agree with the reviewer concerning the reputed flawed experimental quality.

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?
Author response: We have clarified the connection between our results and transgenerational effect in the Discussion. In addition, we have added a sentence concerning the effects of temperature on copepod food requirements to the Discussion. P 15, lines 6-12.

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' CO2 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.
Author response: Here the reviewer is asking about the food quantity, which we have already shown to match the preference of the reviewer. It would be very helpful if the reviewer could have provided an estimate of a sufficient number of animals for prosome length analysis. We realise that the needed number depends on the size of the effect. We managed to find a significant negative $f\mathrm{CO}_2$ effect with 462 adult *Acartia*-females

measured. We have added the possibility of delay in cohort development as a potential reason for the detected effects to the Discussion. P 15, lines 6-12.

p. 18557: Conclusions: The generalization from effects of mineral composition (C/N) to food quality is doubtful.
Author response: We agree that C:N is not the best, or at least not an all-inclusive estimate of copepod food quality. We have added references and this information to the revised manuscript, as well as toned down conclusions based on it. P 4, line 19-21 (Introduction), and P 16, lines 20-24 (Conclusions).

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Ocean acidification challenges copepod phenotypicreproductive plasticity

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Abstract

Ocean acidification is challenging phenotypic plasticity of individuals and populations. Calanoid copepods (zooplankton) are shown to be fairly plastic against altered pH conditions, and laboratory studies indicate that transgenerational effects are one mechanism behind this plasticity. We studied phenotypic plasticity of the copepod *Acartia* sp.*bifilosa* in the course of a pelagic, large-volume mesocosm study that was conducted to investigate ecosystem and biogeochemical responses to ocean acidification. We measured copepod egg production rate,

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egg hatching success, adult female size and adult female antioxidant capacity (ORAC) as a function of acidification (fCO_2 ~365−1231 µatm), and as a function of quantity and quality of their diet. We used an egg transplant experiment to reveal if transgenerational effects can alleviate the possible negative effects of ocean acidification on offspring development. We found significant negative effects of ocean acidification on adult female ~~copepod~~ size ~~and egg hatching success~~. In addition, we found a threshold of fCO_2 concentration (~1000 µatm), above which adaptive maternal effects cannot alleviate the negative effects of acidification on egg hatching and nauplii development. We did not find support for the hypothesis that insufficient food quantity (total particulate carbon < 55 µm) or quality (C:N) weakens the transgenerational effects. However, females with high ORAC produced eggs with high hatching success. Overall, these results indicate that *Acartia* sp. ~~*bifilosa*~~ could be affected by projected near future CO_2 levels.

Keywords: *Acartia bifilosa*, climate change, maternal effects, total particulate carbon, C:N, oxidative stress

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1 Introduction

Increased concentrations of carbon dioxide (CO_2) in the atmosphere is changing the carbon chemistry of the world´s oceans. CO_2 dissolves in seawater thereby decreasing ocean pH. Ocean acidification~~ty~~ is increasing fast and pH is expected to decrease by a further 0.14−0.43 ~~0.43~~ pH units during the coming century (IPCC, 2007). Acidification can cause various problems to biochemical and~~/~~ physiological processes in aquatic organisms. In addition to affecting calcification of calcareous organisms, maintenance of acid-base equilibrium of body fluids may become more difficult and have consequences for example on protein synthesis, metabolism and volume control (Whiteley, 2011).

In a changing environment, populations can respond in three main ways: through plastic responses of individuals, through genetic changes across generations, or through escaping in space or in time by phenology modifications. Under a rapid change, phenotypic plasticity, i.e., the ability of an individual or a population to alter its physiological state, appearance or behaviour in response to the environment is of major importance (West-Eberhard, 2003). Theory predicts that higher plasticity evolves in extreme environments, and that spatial heterogeneity and dispersal select for higher plasticity (Chevin et al., 2013). One could therefore hypothesise that organisms inhabiting a variable environment, such as the study area, could be

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fairly plastic in their response to ocean acidification because they have to cope with both seasonal and sudden changes in pH (~~Brutemark et al., 2011;~~ Almén et al., 2014; Lewis et al., 2013) ~~could be fairly plastic in their response to ocean acidification~~.

Proteomic studies suggest that oxidative stress is a common co-stress of temperature and acidification ~~stress~~ (Tomanek, 2014). Increased production of reactive oxygen species (ROS) may result in increased antioxidant and/or repair costs, and further in reduced investment in reproduction or other functions, such as immune defence. In addition~~Further~~, increased production of ROS may lead to accumulation of oxidative damage and further to acceleration of senescence (Monaghan et al., 2009). There can also be a connection between maternal oxidative balance and offspring quality. In birds, for example, females allocate diverse antioxidants to the eggs that protect the embryo from oxidative stress. This maternal effect has a positive effect on offspring development and growth (Rubolini et al., 2006).

Copepods (zooplankton) are indispensable to the functioning of the whole pelagic ecosystem and contribute significantly to many ecosystem services (Bron et al., 2011). For example, they provide food for early-life stages as well as some adult fishes of many economically important fish species ~~(Beaugrand et al., 2003), as well as some adult fishes such as anchovies and sardines~~ (Steele, 1974; Cushing, 1990~~Alheit and Niquen, 2004~~). ~~In addition, zooplankton graze phytoplankton, and thus participate in controlling harmful algal blooms in the coastal areas suffering from anthropogenic eutrophication (Smayda, 2008).~~

Previous results suggest that calanoid copepods have high buffering capacity against projected ocean acidification for the year 2100 and beyond (Kurihara and Ishimatsu, 2008; Weydmann et al., 2012; McConville et al., 2013; Vehmaa et al., 2013), meaning that they are able to survive, grow, develop and reproduce~~-~~ in lower pH (Reusch, 2014). However, there are also studies showing negative impacts on moderate CO_2 levels (Fitzer et al. 2012), whereas most of the negative impacts have been discovered for extreme, carbon storage scenarios (Kurihara et al., 2004; Mayor et al., 2007; Weydmann et al., 2012). M~~m~~any~~ost of the~~ studies have tested only one life-stage, adult females, and have therefore possibly underestimated the effects of ocean acidification on copepods (Cripps et al., 2014a). There are indications that transgenerational effects are one mechanism responsible for the high plasticity of copepod reproduction against altered pH conditions (Vehmaa et al., 2012). This maternal effect is most likely dependent on the condition of the mother and the availability of food and quality of her diet (Vehmaa et al., 2012; Pedersen et al., 2014a). Paternal effects can also influence offspring traits. Exposure of

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both parents to CO_2 leads to fewer adverse effects on egg production and hatching than exposure of only gravid copepod females (Cripps et al., 2014b). Thor and Dupont (2015) also highlight the importance of testing transgenerational effects. They found significantly lower copepod egg production after two generations when exposed to 900 and 1500 µatm compared to 400 µatm, but transgenerational effects alleviated the negative CO_2 response in 1500 µatm (Thor and Dupont, 2015).

We tested direct and indirect effects of ocean acidification (i.e., via food quantity and quality) on the copepod *Acartia* sp.~~*bifilosa*~~ egg production (EPR), egg hatching success (EH), female body size (measured as prosome length (PL)), as well as antioxidant capacity (ORAC). The study was conducted in association with the KOSMOS (Kiel Off-Shore Mesocosms for Ocean Simulations) project in the Baltic Sea (Paul et al., 2015). The study was intended to cover the low productivity late spring and early summer period, i.e., the post-spring bloom period when pCO_2 concentrations are at the annual minimum. Over the annual cycle, pCO_2 and pH vary substantially at the study site as a result of biological activity and mixing/upwelling of CO_2-enriched deep water (Niemi, 1975; Omstedt et al., 2014). There are also strong spatial gradients in seawater pCO_2/pH, most prominently between the surface layer and the CO_2-rich deeper waters (Almén et al., 2014). Thus, the copepods in the study area are likely to experience strong changes in seawater carbonate chemistry, both seasonally and during their diurnal migration. Total particulate carbon (TPC <55 µm) was used as the measure of food quantity. Food quality was indicated by carbon to nitrogen ratio of the same size fraction of seston (C:N <55 µm) (~~Sterner and Hessen, 1994;~~ Elser and Hasset, 1994; Sterner and Hessen, 1994). In addition, in order to separate transgenerational plasticity (i.e., maternal and paternal effects) and the effect of environment on copepod egg hatching and development, we performed an egg-transplant experiment. Half of the produced eggs were allowed to develop in respective mesocosm water and the other half ~~in the common garden conditions~~ in water collected outside the mesocosm bags.

Due to the high buffering capacity of *Acartia* sp.~~*bifilosa*~~, we hypothesised that there are no fCO_2 related differences in egg production rate, egg hatching success and prosome length between the mesocosms. In addition, ~~W~~we hypothesised that copepod eggs hatch and develop better in the same environment in which they are produced, because transgenerational effects can alleviate the negative effects of ~~females can adjust their reproduction to prevailing conditions~~environmental change. Our third~~second~~ hypothesis stated that low food quantity

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(TPC) and poor quality (high C:N) will weaken the maternal effect by deteriorating the condition of the mother. Finally, we tested if mothers with higher antioxidant capacity (ORAC) produce better quality offspring (EH) by calculating correlation coefficients between the two variables.

2 Materials and Methods

~~This study was conducted in association with the KOSMOS (Kiel Off-Shore Mesocosms for Ocean Simulations) project in the Baltic Sea (Paul et al., 2015).~~ The study was performed in summer 2012 in the vicinity of Tvärminne Zoological Station on the south-western coast of Finland. Six ~~L~~large mesocosms were moored on site in the beginning of June. To enclose the natural plankton community, the mesocosms were left open with only 3 mm mesh size net covering the top and the bottom during filling. After four days, the net was removed and the top was pulled up 1.5 m above the water surface and closed at the bottom (Riebesell et al., 2013; Paul et al., 2015). pH was ~8 and fCO_2 concentrations in the mesocosms prior to adjustment were 237±9 µatm (average±std of daily measurements from all bags). Four mesocosm ~~bags~~ were manipulated with CO_2 enriched seawater, during three consecutive days~~treated with carbon dioxide enriched seawater~~ to reach fCO_2 concentrations of 600-1650 µatm (Paul et al., 2015). Two untreated mesocosms~~bags~~ were used as controls. ~~The~~ The water column was mixed in the beginning of the experiment to avoid salinity stratification. Due to outgassing, CO_2 was also added on day 15 to the upper 7 m of the high CO_2 mesocosms to maintain the treatment levels. No nutrients were added.

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2.1 Sampling

S~~The s~~ampling took place once a week during the first four weeks of the experiment, and once more at the end of the whole experiment ~~, five times~~ (days 3, 10, 17, 24 and 45) ~~during the experiment~~. Mesozooplankton were sampled by taking two hauls with a 300 µm net (17 cm diameter) from 17 m depth and from all ~~the 6~~ mesocosms. The samples were rinsed into containers with 4 l of seawater from respective mesocosm taken from 9 m depth with a water sampler (Limnos, Hydrobios). On the same day, integrated water samples (0-17 m) were collected from all ~~the~~ mesocosms and the Baltic Sea directly into 1.2 l Duran bottles that were closed without head space. Water samples were kept in cool bags and zooplankton samples

were protected from light until transported to a temperature and light controlled room at Tvärminne Zoological Station within 4 h. The light: dark cycle in the room was 16:8 h and light intensity was 7 µmol photons m^{-2} s^{-1} (LI-COR LI-1000). Temperature followed the *in situ* temperature [9°C (day 3), 11°C (day 10), 15°C (day 17), 16°C (days 24 and 45)].

2.2 Measurements of egg production, egg hatching success and prosome length

Twenty adult *Acartia* sp. ~~*bifilosa*~~ (17 females and 3 males) were picked with pipettes from each ~~very~~ sample using stereo microscopes, and gently placed in pre-filled glass bottles with respective mesocosm water. The bottles were closed without head-space, to prevent CO_2-outgassing during the incubation. pH was measured from the bottles before closing ~~them~~ and right after opening them at the end of the incubation using Ecosense pH10 pH/temperature pen (Table S1~~,~~). The pen was calibrated with standard buffer solutions (Certipur, Titripac pH 4.00, 7.00, and 10.00) every second day. The bottles were incubated in ~~the~~ temperature and light controlled room in conditions described above (Materials and Methods 2.1), and mixed three times a day and their place on the shelf was changed randomly. After the incubation (24.3 ± 2.3 h, average ± std), the copepods and produced eggs were filtered using 250 µm and 30 µm sieves, respectively. The copepods were counted and their viability checked before preserving them in RNA*later* (Sigma). RNA*later* can affect size (Foley et al., 2010). The effect depends on the number of segments in the animal, i.e., the more segments the larger effect. Shrinkage is ~15% for copepods (Prof. Elena Gorokhova, Stockholm University, pers. comm.). Prosome length of the preserved female copepods was measured using a stereo microscope (Leica MZ12) and ocular micrometer (total magnification 100 ×). As all the measured copepods were adult females, we assume the shrinkage to be in proportion similar for all individuals, which means that our results are quite conservative. ~~Prosome length of the preserved copepods was measured using a stereomicroscope (Leica MZ12) and ocular micrometer (total magnification 100 ×).~~

In the egg transplant experiment, the collected eggs were divided for hatching into two 50 ml petri dishes with different conditions; one dish was filled with respective mesocosm water and the other filled with Baltic water ~~(common garden)~~. pH of the water was measured as above before the incubations and right after the petri dishes were opened after the incubation (Table S1). The eggs were counted before the petri dishes were completely filled and sealed without head-space using Parafilm. Egg hatching was followed by counting the number of remaining eggs on the dish through the lid using a stereomicroscope twice a day. When the number of

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eggs had remained the same on two consecutive counting times, the dishes were opened and the water containing the remaining eggs and hatched nauplii was preserved with acid Lugol's solution. Therefore the hatching incubation time varied between 63.9 and 137.6 h, depending on incubation temperature. *Acartia* sp. nauplii stages were determined and the number of nauplii and remaining copepod eggs counted using a stereo microscope.

As some adults, copepodites, nauplii or eggs could have ended up in the incubation bottles or petri dishes with the unfiltered incubation water, the egg production rate (EPR, eggs copepod^{-1} d^{-1}) was calculated using only the number of eggs and adult *Acartia* sp. ~~*bifilosa*~~ females found in the incubation bottles after the 24 h incubation. When estimating the egg hatching success (EH, %), the total number of hatched *Acartia* sp. nauplii and remaining eggs at the end of the hatching incubation was compared with the number of eggs counted before the hatching incubation. If the total number exceeded the egg number prior to hatching, the most developed nauplii (>N4) were considered to be carry-over individuals, and were therefore not considered in the estimation of EH. For estimation of nauplii development, rate the development index (DI) was calculated (Knuckey et al., 2005) accordingly,

$$DI = \frac{\sum_{i=0}^{3}(Ni \times ni)}{\sum_{i=0}^{3} ni} \quad (1)$$

where N_i is the assigned stage value (0 for eggs, 1 for N1, 2 for N2 and 3 for N3 and N4) and n_i the number of individuals at that stage. We assume a~~A~~ll the *Acartia* sp. adults and nauplii ~~were considered~~ to be species *A. bifilosa*. However, because~~the~~ another *Acartia* species ~~in the area~~, *A. tonsa* ~~does not usually exist~~ occurs in the area in late summer too~~early June~~ (Katajisto et al., 1998), we cannot be totally sure that we only had one species in the experiments.

2.3 Antioxidant capacity

For antioxidant capacity (ORAC) samples ~25 live female *Acartia* sp. ~~*bifilosa*~~ were picked from every zooplankton sample onto a piece of plankton net in the temperature and light controlled room on days 3, 10, 17 and 31. The net containing the copepods was folded and stored in Eppendorf tubes at -80°C. The samples were homogenised in 150 µl Tris-EDTA buffer containing 1% sarcosyl. The antioxidative capacity was assayed as ORAC ~~according to (~~Ou et al., ~~(~~2001). As a source of peroxyl radicals, ~~we used~~ 2, 2-azobis (2-amidinopropane) dihydrochloride (AAPH) (152.66 mM) was used and fluorescein was used as a fluorescent probe (106 nM). We used trolox (218 µM, Sigma-Aldrich) as a standard and the assay was

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performed on a 96-well microplate and to each well, 20 µL sample, 30 µL AAPH and 150 µL fluorescein wereas added. ORAC values were normalized to protein and expressed as mg Trolox eq. mg protein^{-1}. Protein concentration was measured with NanoOrange® (Life Technologies).

2.4 C:N and TPC

Samples for TPC and C:N were collected onto GF/F filters (Whatman, nominal pore size 0.7 µm) using gentle vacuum filtration (<200 mbar) and then stored in glass petri dishes at -20°C. GF/F filters and petri dishes were combusted at 450°C for 6 hours before use. Gauze pre-filters were used to separate the size fraction < 55 µm. Filters were not acidified to remove inorganic carbon, therefore total particulate carbon is used. C and N concentrations were determined on an elemental analyser (EuroEA) following Sharp (1974), coupled by a Conflo II to a Finnigan Delta^{Plus} mass spectrometer and were used to calculate C:N ratios in mol:mol. For further details on sampling and analyses, please refer to Paul et al. (2015).

2.5 Statistics

The effect of acidification and food quantity and quality on *Acartia* sp. *bifilosa* egg production (EPR), prosome length (PL), antioxidant capacity (ORAC) and nauplii development index (DI) was tested using linear mixed effect models (LMM) with restricted likelihood (REML) approximation from the nlme-package (Pinheiro et al., 2014), where EPR, PL or ORAC were used as response variables, fCO_2, TPC (<55 µm) and C:N as fixed explanatory variables and repeated measure of the mesocosms over time as a random factor (Table 1). Due to the binomial nature of the data, The effect of fCO_2, TPC (<55 µm) and C:N on egg hatching success (EH) was tested with generalized linear mixed model (GLMM) with Laplace likelihood approximation, binomial error structure and logit-link function from the lme4-package (Bates et al., 2014), due to the binomial nature of the data (Table 1). The average of fCO_2, TPC (<55 µm) and C:N measurements from each mesocosm within three days before the zooplankton sampling were used as explanatory variables for EPR, ORAC and EH, because 2–3 days are considered to be an appropriate acclimatisation period for *A. bifilosa* (Yoon et al., 1998; Koski and Kuosa, 1999). For PL, the average of all fCO_2, TPC (<55 µm) and C:N measurements from the start of the mesocosm experiment were used since PL reflects the environmental conditions of the whole lifespan of the animal. In addition, Day 3 was excluded in the LMM testing the PL (Table 1), since three days is too short period forto be able to detecting differences in

copepod size. Egg−adult generation time for *A. bifilosa* at 17°C is approximately 16 days of which ~7.5 d taken by nauplii stages and ~8.5 d by copepodite stages (Yoon et al., 1998). Collinearity between all explanatory variables was checked. Temperature was not considered in the models, because it changed similarly in all the bags (Paul et al., 2015). The model simplifications were done manually in backward stepwise manner by removing the non-significant effects and by using Akaike's information criterion (AIC). We report t- or z-statistics (EH) of the retained fixed effects. To separate the effect of hatching environment from maternal environment, EH and DI were divided with the corresponding values measured in the ~~common garden conditions (~~Baltic Sea water~~)~~. The ratio of Mesocosm EH (or DI) / Baltic~~Common garden~~ EH (or DI) >1 indicates~~means~~ that eggs hatch or develop better in the maternal conditions (Mesocosm water), whereas the ratio <1 indicates~~means~~ that eggs hatch or develop better in the ~~common garden conditions (~~Baltic Sea water~~)~~. The effect of maternal environment (fCO_2, TPC (<55 µm) and C:N) on the ratio was tested with LMM, where the ratio of Mesocosm EH / Baltic~~Common garden~~ EH and Mesocosm DI / Baltic~~Common garden~~ DI were used as response variables; fCO_2, TPC (<55 µm) and C:N as fixed explanatory variables; and repeated measure of the mesocosms over time as a random factor. The model simplifications were made as above.

To test if maternal antioxidant capacity (ORAC) correlates with egg hatching success, Spearman rank correlation tests were used. Data from Days 3, 10 and 17 were included in the test (n = 17, EH result for MC 6 in Day 3 is missing) because those are the days when both ORAC and EH were measured.

All the statistical analyses were performed using software R 3.0.2 (R Core Team, 2013), and~~.~~ the significance level was 0.05.

3 Results

3.1 Egg production, prosome length, antioxidant capacity and egg hatching success

Acartia sp.~~. *bifilosa*~~ egg production (EPR) increased in all mesocosm ~~bag~~s between Day 3 and Day 10, but decreased after that, reaching very low rates (1-2 eggs copepod^{-1} d^{-1}) on Days 24 and 45 (Fig. 1a). Neither food quantity (TPC, <55 µm), food quality (C:N, <55 µm), nor ocean acidification (fCO_2) had a statistically significant e~~a~~ffect~~ed~~ on copepod egg production

~~significantly~~ (Table 2), even though there seemed to be~~were~~ variations in those parameters ~~y~~ ~~differed~~ between the mesocoms (Table 3).

Prosome length (PL) of *Acartia* sp.~~. *bifilosa*~~ females increased during the first week of the study~~,~~; however there seemed to be some differences between the mesocosms already ~~at the start~~ on Day 3, which was not included in the analysis (~~Day 3,~~ Fig. 1b). From Day 10 onwards, the smallest *A. bifilosa* adults were found in the mesocosm with the highest fCO_2 concentration (Fig. 1b). fCO_2, but also TPC (<55 µm) had a statistically significant~~correlated~~ negative~~ly~~ impact on ~~with~~ copepod body size (Table 2).

The overall egg hatching success (EH) was high throughout the study; over 80 % of the *Acartia* sp.~~. *bifilosa*~~ eggs hatched. As seen for EPR, PL, and ORAC, EH also increased from Day 3 to Day 10 in all mesocosms (Fig. 1c). Variance in the EH between the four samplings was highest in the mesocosms with highest fCO_2, whereas EH varied the least and remained >90 % in both control mesocosms (MC1, MC5). ~~Both fCO_2 and~~ In~~De~~spite of this, only TPC (<55 µm) had a statistically significant negative effect~~s~~ on EH (Table 4).

Antioxidant capacity (ORAC) of the female copepods increased from Day 3 to Day 10 in all mesocosms (Fig. 1d). Interestingly, on Day 3 ORAC was highest in the three mesocosms with highest fCO_2 treatment, whereas on Day 31 the situation was opposite and ORAC was lowest i~~o~~n the three mesocosms with highest fCO_2 (Fig. 1d). Despite this, only TPC (<55 µm) had a ~~explained variation in ORAC~~ statistically significantl~~y~~ effect on ORAC; ORAC decreases with increasing TPC (Table 2).

3.2 Egg hatching and nauplii development in mesocosm vs. Baltic Sea conditions~~common garden conditions~~

Neither the maternal food quantity (TPC) nor the quality (C:N) affected the offspring quality (EH and DI) statistically significantly in the egg transplant experiment (Table 5). The fCO_2 was the only detected variable in the maternal environment that influenced the ratio of EH and DI between mesocosm and Baltic~~common garden~~ conditions.

Egg hatching success for eggs hatching in the mesocosm water differed from eggs hatching in the Baltic water~~common garden environment~~. On Days 3 and 10, hatching success was higher in the mesocosm water for the control (MC1, MC5) and for low fCO_2-treatment bags (MC7, MC6), whereas eggs produced in high fCO_2-treatment bags (MC3, MC8) showed higher hatching in the Baltic water~~the common garden conditions~~ (Fig. 2a). Thus, there seems to be a

threshold ~~for~~ fCO_2 for hatching success between 821-1007 uatm~~, above which adaptive maternal effects cannot compensate the negative effects of the environment on offspring development~~. However, on Days 17 and 24 the fCO_2 treatment did not have a clear effect on hatching success. Nevertheless, fCO_2 had a statistically significant negative effect on the ratio of EH mesocosm~~MC~~ / Balk~~k~~tic~~CC~~, meaning that egg hatching was higher in the maternal environment than in the Baltic water when the maternal environment had a low fCO_2 (Table 5). W~~However, w~~hen maternal environment had high fCO_2 the situation was~~is~~ vice versa. The level of fCO_2 had also a significant negative effect on the DI mesocosm ~~MC~~ / Baltic~~CC~~ ratio (Fig. 2b; Table 5).

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3.3 Correlations between antioxidant capacity and offspring quality

Copepod antioxidant capacity (ORAC) was ~~found to~~ correlated significantly with copepod egg hatching success. The relationship between the two variables is positive and stronger for eggs developing in the mesocosm water (rho = 0.75, $p < 0.001$) than for eggs developing in the Baltic water~~common garden environment~~ (rho = 0.62, $p = 0.007$) (Fig. 3).

4 Discussion

In this study, conducted in semi-natural mesocosm environments, reproduction of the ~~copepods~~ *Acartia* sp.~~*bifilosa*~~ copepod showed high phenotypic buffering against acidification, i.e., the species was able to maintain similar egg production rate and also ~~fairly~~ high egg hatching success in all fCO_2 conditions. Nevertheless, we found significant negative effect of ocean acidification on adult female size~~egg hatching success and adult female size~~. Even more interestingly, there seems to be a threshold of fCO_2 concentration (~1000 µatm) for offspring development, above which adaptive maternal effects cannot alleviate the negative effects of acidification on egg hatching and nauplii development (Fig. 2). However, we did not find support for the third~~second~~ hypothesis that poor food quantity (lower TPC) and quality (higher C:N) would weaken the maternal effect by deteriorating the condition of the mother. Conversely, higher food quantity (TPC <55 µm) correlated negatively with egg hatching success, adult female size and antioxidant capacity, whereas C:N ratio did not correlate with any of the measured variables significantly. Copepods were possibly food limited in all the mesocosms, especially after Day 17 due to a sharp decline in Chl *a* concentrations (Paul et al., 2015), and that may have masked the food quality effect. Also, after Day 17 egg production rate was so low that it was practically impossible to find differences in egg production between

the mesocosms. Finally, we found a positive correlation between maternal antioxidant capacity and egg hatching success, suggesting that the female antioxidant defence might also protect the embryo from oxidative stress.

The fact that *Acartia~~.~~* sp.~~*bifilosa*~~ egg production and egg hatching were~~as~~ unaffected by high fCO_2 but egg transplant experiment revealed that development was slower for~~in~~ nauplii at high CO_2 supports the importance of looking beyond egg production and egg hatching, which is also pointed out by Pedersen et al. (2014b). They concluded that the first endogenously feeding nauplii stages of *Calanus finmarchicus* are more sensitive to CO_2-induced acidification than eggs or later nauplii stages (Pedersen et al. 2014b). Longer developmental times in high CO_2/low pH have been observed in crustaceans, echinoderms and molluscs (Cripps et al., 2014a and references therein). Weydmann et al. (2012) also reported a significant developmental delay for *Calanus glacialis* eggs when exposed to highly acidified conditions. Pedersen et al. (2014a) observed that development of C4 copepodites of *C. finmarchicus* was delayed by 8.9 days in high CO_2 treatments in comparison to control condition, when also the previous generation had been exposed to the same conditions.

We expected maternal effects to be most obvious in a high stress situation (high fCO_2 treatments), as seen for three-spined sticklebacks in a study testing the effects of global warming (Shama et al., 2014). Instead, egg hatching was higher and nauplii development faster in the maternal environment than in the Baltic water, when the maternal environment had a low fCO_2 (low stress). In high fCO_2 maternal environment the opposite response was observed, thus indicating that maternal effects are in fact weak and cannot compensate for the higher fCO_2 levels that correspond to near-future levels or that the eggs are damaged by the high fCO_2. This suggests that *Acartia* sp.~~*. bifilosa*~~ and its reproduction are after all somewhat~~fairly~~ sensitive to ocean acidification. However, the effects were not as clear over the following weeks as in the beginning of the study, which may be due to an overall low egg number and large variation in hatching after Day 17, or due to acclimation of the copepods to the treatment conditions. In addition, the maternal effects seemed to weaken over time. This could be due to weakening condition of the mothers. In the absence of fish predators, zooplankton density, and especially *Bosmina* sp. (cladocerans)~~water fleas~~ increased strongly in the mesocosms (Lischka et al., 2015~~current issue~~). Senescence and food limitation were thus plausible problems for copepods, and a likely cause of weakening maternal provisioning. In addition~~Also~~, conditions in the Baltic Sea changed after Day 17 due to an upwelling event, which caused an increase in fCO_2 and

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decrease in pH (Paul et al., 2015). This might have made the Balticcommon garden conditions less favourable for copepod egg development and evened out the differences between high fCO_2 mesocosms and the Balticcommon garden conditions.

A few studies have highlighted the importance of testing for transgenerational effects to avoid over- or underestimation of the effects of ocean acidification on copepods. Similar to our results, Thor and Dupont (2015) found decreasingreduced egg hatching offor *Pseudocalanus acuspes* with increasing pCO_2. In addition, transgenerational effects alleviated the negative effects on egg production and hatching of the second generation when the mothers had been acclimatised to the same treatment. Also, reciprocal transplant experiment showed that the effect was reversible and an expression of phenotypic plasticity (Thor and Dupont, 2015). Contrary to the current studyour results, Pedersen et al. (2014a) found no effect of the CO_2 environment on egg hatching or development of pre-feeding nauplii stages N1 and N2 in their multigenerational study using *C. finmarchicus*. However, the development time of larger nauplii and copepodite stages was increased by pCO_2, although the development delay was not detected anymore in the nextfollowing generation (Pedersen et al., 2014a). Vehmaa et al. (2012) studied combined effects of ocean acidification and warming, and found indications that negative effects on *Acartia* sp.*bifilosa* reproductive success can partly be partly combated with maternal effects. THowever, the used pH treatments (-0.4 from ambient) were aton the same level aswith the low fCO_2-treatments in this study (MC6, MC7), which makes the results of the two studies consistent.

The measurements of female copepod antioxidant capacity were done in order to provide possible additional information of the maternal provisioning on the offspring. A preferable practice in oxidative stress studies is to measure several of the four components consisting of free radical production, antioxidant defences, oxidative damage, and repair mechanisms (Monaghan et al., 2009). In the current study we only have the estimate for the defences, antioxidant capacity (ORAC) measurements, which makes our conclusions slightly more uncertain. However, an earlier study with the same species has indicated that at intermediate stress levels an upregulation of the antioxidant system enhances protection against oxidative damage, but at higher stress, the pro-oxidants may exceed the capacity of the antioxidant system and lead to oxidative damage (Vehmaa et al., 2013). In this study, upregulated antioxidant defence seemed to have a positive effect on offspring quality, as indicated by the positive correlation between female ORAC and egg hatching success. The slightly higher correlation in

~~the mesocosms environment compared to the common garden conditions indicates that the female can provision her eggs to match the prevailing conditions.~~ Higher ORAC in the two highest fCO_2 mesocosms in the beginning of the study could be a sign of an upregulated antioxidant system in a sudden stressful situation, whereas the lowest ORAC in the high fCO_2 treatments at day 31 (Fig. 1d~~D~~) could be caused by prolonged stress and exhausted antioxidant defence. The change from positive to negative effect in the course of the study could explain why fCO_2 did not show a significant correlation with ORAC, whereas food quantity (TPC <55 µm) did.

Ismar et al. (2008) showed that *Acartia* spp. development can be either slow or altered by certain algal groups causing death before the first copepodite or reproductive stage. A non-optimal diet could explain the observed contradictory effects of TPC. It is hard to explain why higher food quantity would otherwise cause smaller adult female size, lower egg hatching success or lower antioxidant capacity, unless it is nutritionally unbalanced or difficult to catch or assimilate. Since we did not study what the copepods preyed upon~~were consuming~~ we can only speculate on diet quantity and quality. Satiated food conditions can strengthen the maternal or transgenerational effects. The transgenerational effects were of minor importance for hatching success in *C. finmarchicus* when exposed to long term high CO_2 and food limited conditions (Pedersen et al., 2014a). Long term stress and food limitation could thus also be the reason for weakening maternal effects in the current study.

We found body size (prosome length) to be negatively affected by high CO_2. The result seems to be mostly driven by the mesocosm with the highest fCO_2 (MC 8), where the adult *Acartia*~~.~~ sp.~~*bifilosa*~~ copepods were smallest on all the four sampling times that were included in the analysis (Days 10, 17, 24 and 45) (Fig. 1b). I~~Since i~~t 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 If *Acartia sp.* development follows~~According to the Bělehrádek's temperature function it ~~would~~ takes 12−15 days for VI 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 used in Dzierzbicka-Glowacka et al. (2009) for *A. bifilosa*. ~~is plausible that at 9−11°C.~~ It is thus possible that the copepods could have ~~also~~ developed through several stages causing the differences in prosome length between the treatments on Day 10. Lowered pH may have increased copepods´ energy requirements and if energy is reallocated towards maintaining homeostasis, their somatic growth can be reduced. Pedersen et al. (2014a) found *C.*

finmarchicus body size to be inversely related to pCO_2. They also found higher respiration rate under more acidified conditions, and claimed that increased energy expenditure via rising respiration and consecutive decreasing growth and reproduction could lower the energy transfer to higher trophic levels and thus hamper the productivity of the whole ecosystem (Pedersen et al., 2014a). This is especially alarming when considering the projected climate warming, since copepod size is negatively correlated with temperature (Foster et al., 2011). In addition to temperature, ~~also~~ food quantity and quality can affect the copepod body size (Hart and Bychek, 2011). T~~Also, t~~emperature and food also interact because temperature affects the respiration and metabolism, thus the satisfying diet depends on temperature (Boersma et al., 2016). If high CO_2 treatment (MC 8) caused a developmental delay in maturation, as could be interpreted from the prosome length results (Fig. 1b), the maturation would have occurred at different temperature than in other mesocosms and possibly in non-optimal food conditions. ~~In addition to temperature, also food quantity and quality can affect the copepod body size (Hart and Bychek, 2011).~~ ~~;~~ ~~h~~AnywayH~~owever~~, higher food quantity and quality would be~~the~~ expected to increase copepod size~~effect would be positive~~, contrary to our results. It is therefore possible that the used food quantity (TPC <55 µm) and quality estimates (C:N <55 µm) do not fully describe the diet that *Acartia*~~.~~ sp.~~*bifilosa*~~ was consuming in the mesocosm ~~bag~~s.

Adult copepods have in general shown robustness against acidification (Mayor et al., 2012, McConville et al., 2013), whereas eggs and nauplii appear to be more sensitive (Cripps et al., 2014b~~;~~, Fitzer et al., 2012). In addition, there seems to be notable differences in sensitivity between species. Nauplii production, adult female fatty acid content and antioxidant capacity (ORAC) of *Eurytemora affinis* were not affected by fCO_2 in the current mesocosm campaign (Almén et al., 2016~~5~~). Similarly, Lewis et al. (2013) found differences in ocean acidification sensitivity between the species *Oithona similis* and *Calanus* spp. (*C. glacialis* and *C. hyperboreus)*. They argued that *O. similis* is more sensitive~~less adapted~~ to future ocean acidification than *Calanus spp.*~~. *glacialis* to a narrower range of pH~~, because *O. similis* remains in the surface waters whereas *Calanus* spp. migrates vertically, and encounters~~crosses~~ a lot wider pCO_2 ranges daily than *O. similis*~~of less pronounced vertical migration patterns~~ (Lewis et al., 2013). The same applies to *Acartia* sp.~~*bifilosa*~~ and *E. affinis* in our study area. Although *Acartia* spp.~~*bifilosa*~~ is exposed to natural variability in pH environment due to daily variations as well as due to staying at greater depths during the day (low pH in deep water), it does not reside as deep ~~down~~ as *E. affinis* (Almén et al., 2014) and may therefore show higher sensitivity than *E. affinis* during the current mesocosm campaign (Almén et al., 2016~~5~~).

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The results obtained for *Acartia* sp.~~ *bifilosa*~~ reproduction in the current study seem to contradict the results obtained for the *Acartia* sp.~~ *bifilosa*~~ abundance ~~determined in the mesocosm bags~~. Although our results indicate that *Acartia* sp.~~ *bifilosa*~~ reproduction ~~is~~ in fact sensitive to ocean acidification, no fCO_2 effect was found for the abundance of this species (Lischka et al., ~~current issue~~2015). It is possible that 45 days was not long enough to detect small negative effects of CO_2 on copepod size, egg hatching and nauplii development, to be reflected in copepod abundance. In addition, especially in the beginning of the study ~~*A*~~*cartia* ~~eggs in the mesocosms~~ might have ended up in the sediment trap before hatching due to slow development at low temperature, which might have made it difficult to detect differences in ~~*A*~~*cartia* ~~abundance~~ between the mesocosms. On a longer time scale,~~ however,~~ small acidification induced delays in offspring development~~these~~ could translate into negative effects for the copepod population, and further on energy transfer within the pelagic food web. In addition, warming will probably enhance the sensitivity of the species towards ocean acidification (Vehmaa et al., 2012, 2013).

5 Conclusions

Our results support the idea that it is important to look beyond egg production as hatching and development can be more sensitive to ocean acidification. Parental effects will likely be important in mediating some of the negative effects of ocean acidification. For *Acartia* sp.~~ *bifilosa*~~, the transgenerational (maternal) effects may alleviate negative impacts of ocean acidification but only under exposure to medium levels of CO_2. We did not find support for the hypothesis suggesting that poorer food quantity and quality would weaken the maternal effect by deteriorating the condition of the mother, which could be due to the overall food limitation especially during the latter half of the study or the fact that our estimates of food quantity and quality did not describe the diet in a satisfactory manner. Nevertheless, maternal antioxidant defence seems to correlate positively with offspring egg hatching success. Overall, these results indicate that *Acartia* sp.~~ *bifilosa*~~ could in fact be affected by CO_2 levels predicted for the year 2100 (IPCC, 2007). However, it is important to remember that this study shows how today's copepods would react to tomorrow's world; thus these results do not take into account the possible effects of evolutionary adaptation. Transgenerational effects can buffer short-term detrimental effects of ocean acidification and thus give time for genetic adaptation and consequently assist persistence of populations under climate change.

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Author contributions

A.V. planned the experiment; A.V., A.-K.A., J.E.-Ö., A.B. conducted the laboratory experiment; A.V. performed the statistical analyses; A.P. analysed TPC and C:N; S.F analysed ORAC; U.R. coordinated the whole project; A.V. and A.-K.A. shared responsibility of writing the manuscript with contributions from all co-authors..

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Tables

Table 1. The structure of ~~Variables that were used in~~ the full LMM or GLMM models that were used to test effects of ocean acidification, food quantity, and food quality on copepod egg production (EPR), egg hatching success (EH), prosome length (PL), antioxidant capacity (ORAC), the ratio of EH mesocosm / EH Baltic~~common garden~~, and the ratio of nauplii development index (DI) mesocosm / DI Baltic~~common garden~~. T~~, their definitions and t~~he sampling days that were included in each of the models are listed. Repeated measures of same mesocosm bags was used as a random effect in all the models, because copepods that come from the same bags are more alike than copepods from different bags.

~~Variable type~~	~~Variable~~	~~Definition~~	~~Days~~	~~3~~	~~10~~	~~17~~	~~24~~	~~31~~	~~45~~
~~Fixed effects~~	~~fCO_2~~	~~The ocean acidification effect~~	~~EPR (LMM)~~	~~x~~	~~x~~	~~x~~	~~x~~		~~x~~
	~~TPC <55 µm~~	~~The food quantity effect~~	~~EH (GLMM)~~	~~x~~	~~x~~	~~x~~	~~x~~		
	~~C:N <55 µm~~	~~The food quality effect~~	~~PL (LMM)~~		~~x~~	~~x~~	~~x~~		~~x~~
~~Random effects~~	~~Repeated measures of same mesocosm bags~~	~~Copepods that come from the same bags are more alike than copepods from different bags~~	~~ORAC (LMM)~~	~~x~~	~~x~~	~~x~~		~~x~~	
			~~EH MC / CG~~ ~~DI MC / CG~~ ~~(LMM)~~	~~x~~	~~x~~	~~x~~	~~x~~		

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Response variable	Fixed effects	Effect tested	Days included in the model					
			3	10	17	24	31	45
EPR (LMM)	fCO_2	Ocean acidification	x	x	x	x		x
	TPC (<55 µm)	Food quantity						
	C:N (<55 µm)	Food quality						
EH (GLMM)	fCO_2	Ocean acidification	x	x	x	x		
	TPC (<55 µm)	Food quantity						
	C:N (<55 µm)	Food quality						
PL (LMM)	fCO_2	Ocean acidification		x	x	x		x
	TPC (<55 µm)	Food quantity						
	C:N (<55 µm)	Food quality						
ORAC (LMM)	fCO_2	Ocean acidification	x	x	x		x	
	TPC (<55 µm)	Food quantity						
	C:N (<55 µm)	Food quality						
EH MC/Baltic (LMM)	fCO_2	Ocean acidification	x	x	x	x		
	TPC (<55 µm)	Food quantity						
	C:N (<55 µm)	Food quality						
DI MC/Baltic (LMM)	fCO_2	Ocean acidification	x	x	x	x		
	TPC (<55 µm)	Food quantity						
	C:N (<55 µm)	Food quality						

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Table 2. T-statistics of the retained fixed effects in the linear mixed effect models ~~LMMs~~testing the effects of TPC (<55µm), C:N and ~~*f*CO$_2$ on egg production rate (EPR), female prosome~~ length (PL) and female antioxidant capacity (ORAC). Repeated measures of same mesocosm bags was used as a random effect in all the models, because copepods that come from the same bags are more alike than copepods from different bags.

~~5.1.1.1.1.1.1.1~~ Response variable	Fixed effect~~Variable~~	Estimate~~Value~~	DF	t	p-value
EPR	TPC <55 µm	0.21±0.14	23	1.54	0.137
~~TPC <55 µm~~	~~0.21±0.14~~	~~23~~	~~1.54~~	~~0.137~~	
PL	*f*CO$_2$	-0.000027±0.000011	16	-2.39	0.030
~~*f*CO$_2$~~	~~-0.000027±0.000011~~	~~16~~	~~-2.39~~	~~0.030~~	
~~TPC <55 µm~~	TPC <55 µm	-0.0037±0.0017	16	-2.21	0.042
ORAC	TPC <55 µm	-0.0045±0.0021	22	-2.17	0.041
~~TPC <55 µm~~	~~-0.0045±0.0021~~	~~22~~	~~-2.17~~	~~0.041~~	

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Table 3. Ranges of fCO_2, TPC<55 µm, and C:N< 55 µm that were used as explanatory variables in the full LMM and GLMM models. 3-day averages (measured within the latest three days of the sampling day) were used in testing the effects of the explanatory variables on copepod egg production (EPR), antioxidant capacity (ORAC), and egg hatching success (EH), whereas average of all measurements sincefrom the start of the experiments until the sampling day were used when testing the effects of the explanatory variables on copepod size (PL). Variations in fCO_2, TPC <55 µm, and C: <55 µm in the course of the study are presented in Paul et al. (2015).

	fCO_2 (µatm)		TPC<55 µm		C:N <55 µm	
	3-d average	Average since Day 1start	3-d average	Average since Day 1start	3-d average	Average since Day 1start
MC 1	267–477	267–365	15.1–31.6	21.4–31.6	5.51–8.43	7.26–8.03
MC 3	745–1201	884–1121	17.4–29.7	20.4–29.7	6.94–8.36	7.79–8.20
MC 5	275–481	274–368	15.8–24.5	19.2–24.8	7.24–8.57	7.24–7.59
MC 6	663–991	683–896	16.5–34.3	21.0–34.3	7.14–8.25	7.60–7.81
MC 7	390–565	390–497	17.5–30.0	21.4–29.9	6.92–8.25	7.43–7.74
MC 8	874–1525	1117–1413	17.4–26.3	21.6–26.3	7.16–8.53	7.59–7.93

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Table 4. Z-statistics of the retained fixed effects in the GLMM testing the effect of fCO_2, TPC (<55 µm) and C:N on egg hatching success (EH). Repeated measures of same mesocosm bags was used as a random effect in the model, because copepods that come from the same bags are more alike than copepods from different bags.

~~5.1.1.1.1.2~~ Response variable	Fixed effect	Estimate~~Value~~	z	p-value
~~EH~~				
EH fCO_2	fCO_2	-0.00062±0.00032	1.94	0.052
~~TPC <55 µm~~	TPC <55 µm	-0.09557±0.02505	3.82	<0.001

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Table 5. T-statistics of the retained fixed effects in the LMMs testing the effect ~~of~~ fCO_2, TPC (<55 µm) and C:N on ratio of egg hatching success (EH) mesocosm / EH Baltic~~common garden~~ and nauplii development index (DI) mesocosm / DI Baltic~~common garden~~. Ratio >1: higher EH or DI in the mesocosm water (maternal environment) than in the Baltic Sea water ~~(common garden environment)~~, ratio <1: lower EH or DI in the mesocosm water (maternal environment) than in the Baltic Sea water ~~(common garden environment)~~. Repeated measures of same mesocosm bags was used as a random effect in both models, because copepods that come from the same bags are more alike than copepods from different bags.

~~5.1.1.1.1.3~~ Response variable	~~5.1.1.1.1.4~~ Estimate~~Value~~ ixed effect	DF	t	p-value
	~~EH mesocosm / EH common garden~~			
EH mesocosm / EH Baltic~~common garden~~	fCO_2	-0.000061±0.000028	16	-2.20~~3~~	0.043
DI mesocosm / DI Baltic~~common garden~~	fCO_2	-0.000145±0.000067	16	-2.15	0.047
	~~DI mesocosm / DI common garden~~			
	~~fCO_2~~	~~-0.000145±0.000067~~	~~16~~	~~-2.15~~	~~0.047~~

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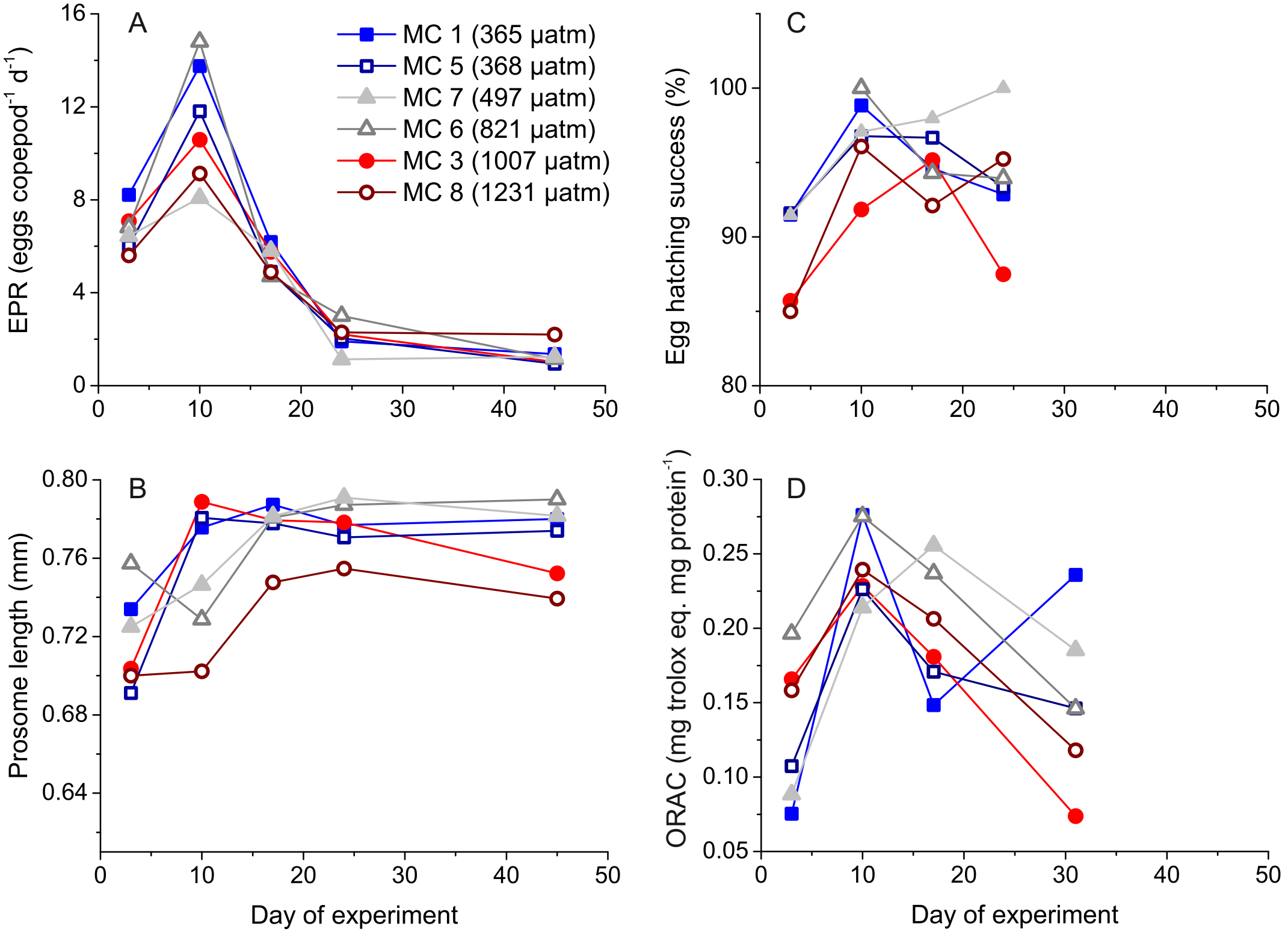
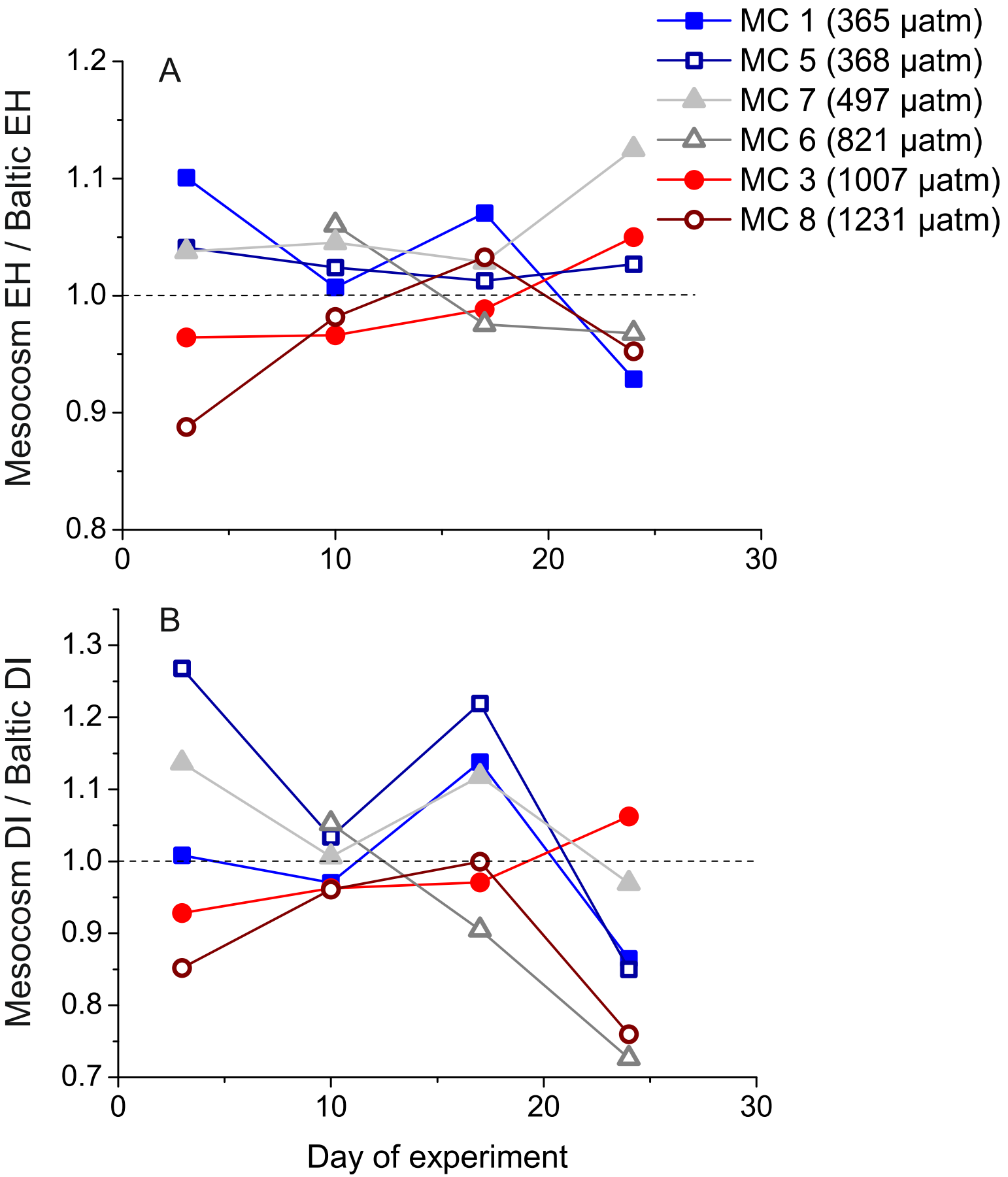
Figures.

Fig. 1. Development of *Acartia bifilosa* a) egg production, b) prosome length, c) egg hatching success, and d) antioxidant capacity in the mesocosms in the course of the study. The fCO_2 (µatm) values represent the average in Days 1−43 (Paul et al., 2015).

Fig. 2. Development of the ratio of a) egg hatching success (EH) mesocosm / EH Baltic~~common garden~~ and b) nauplii development index (DI) mesocosm / DI Baltic~~common garden~~ during the study. Ratio >1: higher EH or DI in the mesocosm water (maternal environment) than in the Baltic Sea water ~~(common garden environment)~~, ratio <1: lower EH or DI in the mesocosm water (maternal environment) than in the Baltic Sea water ~~(common garden environment)~~. Note that because of different development times, the DI values are not comparable between the days. The fCO_2 (µatm) values represent the average in Days 1−43 (Paul et al., 2015).

Fig. 3. Correlations of copepod egg hatching success (EH) with maternal antioxidant capacity (ORAC).


A
B
C
D
MC 1 (365 µatm)
MC 5 (368 µatm)
MC 7 (497 µatm)
MC 6 (821 µatm)
MC 3 (1007 µatm)
MC 8 (1231 µatm)
EPR (eggs copepod-1 d-1)
Egg hatching success (%)
Prosome length (mm)
ORAC (mg trolox eq. mg protein-1)
Day of experiment
Day of experiment


MC 1 (365 µatm)
MC 5 (368 µatm)
MC 7 (497 µatm)
MC 6 (821 µatm)
MC 3 (1007 µatm)
MC 8 (1231 µatm)
A
Mesocosm EH / Baltic EH
1.2
1.1
1.0
0.9
0.8
0
10
20
30
B
Mesocosm DI / Baltic DI
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0
10
20
30
Day of experiment

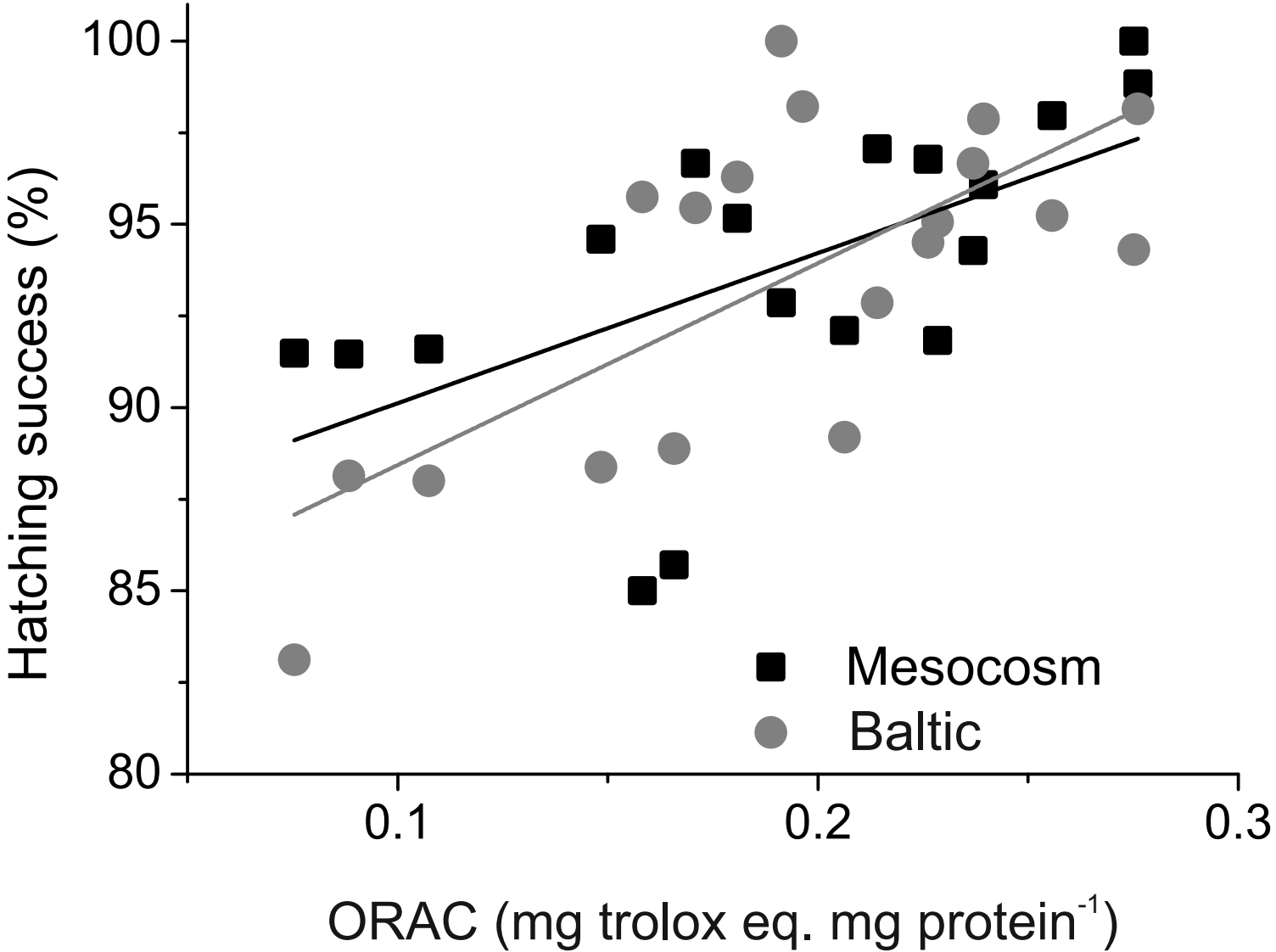

Hatching success (%)
100
95
90
85
80
0.1
0.2
0.3
ORAC (mg trolox eq. mg protein-1)
Mesocosm
Baltic

Table S1. pH values measured before and after the incubations. After incubation pH values for the Baltic are averaged from six measurements.

	pH before – after incubation								
	Day 3		**Day 10**		**Day 17**		**Day 24**		**Day 45**
	EPR, PL	**EH, DI**	**EPR, PL**	**EH, DI**	**EPR, PL**	**EH, DI**	**EPR, PL**	**EH, DI**	**EPR, PL**
MC 1	8.12-8.13	7.97-7.51	8.14-8.18	8.2-7.95	8.08-8.11	8.10-7.96	7.98-8.07	8.05-7.92	7.82-7.82
MC 3	7.50-7.63	7.62-7.61	7.62-7.66	7.67-7.81	7.61-7.64	7.64-7.68	7.59-7.69	7.69-7.72	7.69-7.69
MC 5	8.18-8.07	8.08-7.79	8.10-8.17	8.16-8.00	8.07-8.08	8.10-7.83	7.90-8.00	8.01-7.84	7.85-7.89
MC 6	7.66-7.71	7.66-7.67	7.63-7.73	7.73-7.87	7.72-7.78	7.73-7.69	7.63-7.77	7.76-7.72	7.75-7.73
MC 7	7.97-7.85	7.82-7.69	7.90-7.97	7.95-7.90	7.91-7.94	7.89-7.74	7.84-7.88	7.90-7.82	7.82-7.84
MC 8	7.45-7.59	7.50-7.58	7.53-7.61	7.57-7.74	7.58-7.58	7.59-7.60	7.50-7.65	7.64-7.68	7.62-7.68
Baltic	8.15	7.92-7.68	8.24	7.9-7.95	8.36	7.90-7.90	8.08	7.88-7.84	7.67

Table S2. Number of nauplii hatched / total number of eggs incubated, as well as nauplii development index (DI) in the egg transplant experiment.

Mesocosm	**Hatching conditions**	**Day 3**		**Day 10**		**Day 17**		**Day 24**	
		nauplii / total eggs	**DI**	**nauplii / total eggs**	**DI**	**nauplii / total eggs**	**DI**	**nauplii / total eggs**	**DI**
MC 1	**MC**	43 / 47	1.02	85 / 86	0.99	35 / 37	1.27	26 / 28	1.43
	Baltic	64 / 77	1.01	53 / 54	1.02	76 / 86	1.12	23 / 23	1.65
MC 3	**MC**	60 / 70	1.10	45 / 49	0.94	59 / 62	1.10	21 / 24	1.42
	Baltic	48 / 54	1.19	77 / 81	0.98	52 / 54	1.13	20 / 24	1.33
MC 5	**MC**	109 / 119	1.47	60 / 62	1.00	29 / 30	1.53	14 / 15	1.13
	Baltic	22 / 25	1.16	86 / 91	0.97	63 / 66	1.26	30 / 33	1.33
MC 6	**MC**	na	na	52 / 52	1.02	33 / 35	1.09	31 / 33	1.30
	Baltic	55 / 56	1.50	149 / 158	0.97	29 / 30	1.20	33 / 34	1.79
MC 7	**MC**	75 / 82	1.33	66 / 68	0.97	48 / 49	1.22	11 / 11	1.46
	Baltic	52 / 59	1.17	52 / 56	0.96	40 / 42	1.10	16 / 18	1.50
MC 8	**MC**	51 / 60	1.03	49 / 51	0.96	35 / 38	1.03	20 / 21	1.24
	Baltic	45 / 47	1.21	92 / 94	1.00	33 / 37	1.03	27 / 27	1.63