Biogeosciences Discuss., 12, C9052–C9057, 2016
www.biogeosciences-discuss.net/12/C9052/2016/
© Author(s) 2016. This work is distributed under
the Creative Commons Attribute 3.0 License.

Biogeosciences Discussions
Open Access
EGU



# *Interactive comment on* "Negligible effects of ocean acidification on *Eurytemora affinis* (Copepoda) offspring production" *by* A.-K. Almén et al.

**A.-K. Almén et al.**

Jonna.Engstrom-Ost@novia.fi

Received and published: 14 January 2016

Response to comments by Referee #2

We thank for the constructive comments on our manuscript. We considered all comments and suggestions when revising the manuscript. Below we have responded with our comments and description of changes made to the manuscript.

Introduction: Comment 1, Referee #2, P. 17096 L. 29, A more appropriate reference could be used here rather than Riebesell and Tortell e.g., Schoo et al 2013.

Author response: The reference has been changed to Schoo et al., 2013.

Comment 2, Referee #2, P. 1098 L. 22, please can you put in the deviation with these averaged fCO2 values.

Author response: The standard deviation for the fCO2 values cannot be calculated as we did not have replicates of the mesocosms. There was a slight variation over time as the enclosures were allowed to vary naturally, except for one addition of CO2 at t15; however, there was a clear difference between treatments during the whole experiment. How fCO2 varied over time is described in the overview paper by Paul et al. (2015).

Methods: Comment 3, Referee #2, P. 17099 L. 8, Were the females incubated individually with 10 replicates, or were there 10 individuals per replicate? If the latter applies, how many replicates were used?

Author response: There were 10 individuals per bottle/replicate and one bottle per treatment. The experiment was repeated four times, i.e. we used repeated measurements from the same groups of individuals. This was considered in the statistical analyses, linear mixed effects models (LMM) with random structure that takes into account these dependencies.

Comment 4, Referee #4, P. 17099 L. 28, Why were only first stage nauplii included in the analysis? If all nauplii were filtered out and preserved daily, then nauplii beyond stage 1 should be counted as these too would have been produced from the females over the preceding 24 hours.

Author response: Only first stage nauplii were counted. If we consider the inter-clutch time and production of a new egg sac, the hatching and development of the nauplii would not have had time to reach N2 (second stage nauplii) within 24h. The development time in E. affinis is approximately 1 day per stage at 14 °C (Devreker et al., 2012), and the incubation temperature in the current work was ∼10-15°C. Any nauplius beyond the first stage could therefore have been introduced with the water and not hatched from the incubated females. Only a few second stage nauplii, in total, were observed in the samples.



BGD

12, C9052–C9057, 2016



CC BY

Comment 5, Referee #2, P. 17102 L 4: spelling error “fort”

Author response: Spelling error corrected

Comment 6, referee #2, Line 25: did you analyse the fatty acid response of the eggs to the pH? If so, please produce the results. If not, perhaps this should be done to determine a secondary effect of pH on female reproduction, or indeed a direct response of pH on the eggs.

Author response: We found no effect of pH on the fatty acid levels of the eggs. We however chose not to include this analysis and associated results in the manuscript at this stage, as it is highly unlikely that there would be a direct effect of CO2 on the newly protruded eggs. Neither nauplii production, nor female fatty acids were affected, whereas fatty acids of females affected fatty acids of their eggs.

Comment 7, Referee #2, Page 17103 Line 11: What did you plot the standardized residuals against? fitted values? Author response: P. 17103 L. 11 The standardized residuals were plotted against the fitted values. This is added on L. 11. Results:

Comment 8, Referee #2, For Figures 2, 3 and 4 please put in correlations (R2), significance (p-value) and equations on the graphs or in the legends. For Figure 1, please add in the standard deviations. In Figure 2b, there are a few outliers, did this not influence the LMM? In other words, was the variance structure in the standardized residuals of this model valid?

Author response: Figure 2 presents the relationship between daily nauplii production and Chl a or diatom concentration. The graph includes repeated measures of the same groups of individuals over four days, and repeated measurements of the same mesocosms over four weeks. That is why we used linear mixed effects models (LMM) with random structure that takes into account these dependencies to analyse the dataset. We therefore cannot add correlation nor linear regression results to the figure legends. Statistical results corresponding to the figures are reported in Table 3. The same ap-



BGD

12, C9052–C9057, 2016



CC BY

plies for Figures 3 and 4 (weekly, repeated measures of female fatty acid levels from the same mesocosms and weekly averaged of female nauplii production analysed with LMM). Figure 1. Please notice that the average values in Figure 1 are averages of nauplii production calculated from the total amount of nauplii per bottle divided by the number of live females per bottle, so standard deviation can unfortunately not be applied here.

Fig 2b. We thank the reviewer for pointing this out. Data shown in Figure 2b was analysed with LMM where other variables were included also. The diatoms did not influence the model negatively. However, while rerunning our statistical analyses, with log-transformation to get a better model fit, we also discovered that dinoflagellates were significant for the number of nauplii produced. Dinoflagellates had a positive effect on the nauplii production. Therefore, we have updated our manuscript including this new result, as well as shortly discussed dinoflagellates and their effects under section 4.2. The overall results are the same for the other variables.

Discussion: Comment 9, Referee #2, P. 17104, L. 5. can you add in the natural variability in pH/fCO2 experienced by the copepods on a daily basis in your area of research. I think this would be a strong addition to this argument.

Author response: In the study area a previous study (Almén et al. 2014) showed that copepods experience changes in pH of up to 0.5 units within 24h during summer (7.51-8.1). We have now added this information in the discussion.

In addition we removed the regression lines from figure 2-4 as they seem to confuse the reader which statistical method we used. We also corrected the title in Figure 4 as well as rewrote the Figure captions to improve the explanation concerning which method were used for analyses. We considered Bonferroni correction (Table 3), but it is not necessary in the analyses applied here, as they do not answer the same hypotheses, the correction was removed from the results and Table 3 (Personal communication with departmental statistician, Åbo Akademi University). The results remain the same for



BGD
12, C9052–C9057, 2016

everything else, except model 8 (MUFA, p = 0.032). The group of autotrophic dinoflagellates was renamed to autotrophic/mixotrophic dinoflagellates and their size range (10-100 $\mu$m) was included. The group contains Dinophysis spp. Microcanthodinium, Amylax and Heterocapsa triquetra. This piece of information has been added to the methods section of the manuscript.

References used in the responses to referees #1 and #2:

Almén, A-K., Vehmaa, A., Brutemark, A., and Engström-Öst, J.: Coping with climate change? Copepods experience variation in their physicochemical environment on a diurnal basis. J. Exp. Mar. Biol. Ecol., 460, 120–128, 2014.

Devreker D., Pierson, J. J., Soussi, S., Kimmel, D. G., and Roman, M. R.: An experimental approach to estimate egg production and development rate of the calanoid copepod Eurytemora affinis in Chesapeake Bay, USA. Journal of Experimental Marine Biology and Ecology 416-417, 72–83, 2012.

Devreker, D., Souissi, S., Forget-Leray, J., and Leboulenger, F.: Effects of salinity and temperature on the post-embryonic development of Eurytemora affinis (Copepoda;Calanoida) from the Seine estuary: a laboratory study. J. Plankton Res. 29 (suppl 1), i117–i133, 2007.

Paul, A. J, Bach L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Hellemann, D., Trense, Y. Nausch, M. Sswat, M., and Riebesell, U.: Effect of elevated CO2 on organic matter pools and fluxes in a summer, post spring-bloom Baltic Sea plankton community. Biogeosciences, 12, 6181–6203, doi: 10.5194/bg-12-6181-2015, 2015.

Schoo, K. L., Malzahn, A. M., Krause, E., and Boersma, M.: Increased carbon dioxide availability alters phytoplankton stoichiometry and affects carbon cycling and growth of a marine planktonic herbivore. Mar. Biol. 160:2145–2155 DOI 10.1007/s00227-012-2121-4, 2013.

Interactive comment on Biogeosciences Discuss., 12, 17093, 2015.