

## ***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 #1

We thank Referee #1 for the constructive comments on our manuscript. We have considered all comments and suggestions when revising the manuscript. Please see response below:

Comment 1, Referee #1, P.17098 L.26: Please, give a good reason for choosing these dates to perform the egg production experiments. Author response: The dates for the sampling were chosen according to the sampling procedure of the mesocosms, agreed

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upon by all participating researchers (Paul et al., 2015). Sample volumes and number of net tows for zooplankton were restricted and had to be shared amongst participants of the campaign. The sampling was conducted in collaboration with other research groups. We started our experiments on the days we received zooplankton samples, which was once per week and continued the experiment during the days we received water samples, collected from the mesocosms.

Comment 2, Referee #1, P. 17099 L3. Give an explanation for why you didn't filter the incubation water to avoid in this way the other predators or nauplii produced from other species. Author response: We did not filter the water in order to keep food conditions as similar to in situ conditions as possible. Another factor that is affected by filtering is gas exchange which would have affected the pH conditions. We avoided also this problem by not filtering. To minimize handling of the restricted amount of water available, the water was transferred directly from the samplers to the incubation bottles. We do realize that the probability of introducing other zooplankton would have been decreased using filtration.

Comment 3, Referee #1, P. 17100 L. 27. – P. 17101 L. 12 Many methodological details for parameters (carbon and nitrogen concentrations, phytoplankton) that they are not presented neither in the results nor in the discussion. Author response: The methods on these parameters have now been shortened and we instead provide a reference for the more detailed method description in Paul et al. (this issue).

Comment 4, Referee #1, P.17101 L.14. The authors have used C:N, dinoflagellates and other parameters for their statistical analysis however there is no any information or relative reference in this manuscript how these parameters changed over time and with the different pCO<sub>2</sub> levels. I would be easier to follow the results and the discussion if the authors provide this information. Author response: We have included a short description of the C:N and other parameters from our sampling days on P. 17103 L. 24. C:N <55 $\mu$ m was not affected by CO<sub>2</sub>. The C:N values included in our analyses (our sampling days) were on average 7.66 $\pm$ 0.42 (range 6.13-8.77). Autotrophic dinoflag-

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ellates were on average  $4.41 \pm 1.39 \mu\text{g C L}^{-1}$  (range 0-7.32) and declined rapidly after t17. For a more comprehensive description of C:N please refer to Paul et al. (2015). Comment 5, Referee #1, P. 17102 L. 4, Correct "fort" to "for". Author response: Spelling mistake corrected

Comment 6, Referee #1, P.17104 L.5. The authors don't No discuss at all these two parameters and if they changed with the elevated CO<sub>2</sub> or not. Please explain the reason. Author response: The biomass of autotrophic dinoflagellates and particulate matter C:N did not change significantly with CO<sub>2</sub>. A description of how C:N <55 μm varied in the mesocosms over time can be found in Paul et al. (2015). Likewise the chlorophyll a dynamics is explained in the overview paper by Paul et al. (this issue) and therefore it is not described in this manuscript to avoid overlapping. Autotrophic dinoflagellates were on average  $4.41 \pm 1.39 \mu\text{g C L}^{-1}$  and declined after t17 and there was no effect of CO<sub>2</sub>. We have focused the discussion on the variables retained in the LMM model that are the ones that affected nauplii production.

Comment 7, Referee #1, P. 17106 L. 4, "The abundance of diatoms was high during the first days but then declined rapidly" How and where did you show this in the manuscript. Please clarify how the estimated parameters changed during your experiments. Author response: We have included a short explanation on how the parameters varied over time. The diatoms included in our analyses ranged from 0-7.32 μg C L<sup>-1</sup> (average  $0.06 \pm 0.1$ ).

Comment 8, Referee #1, L.14-16. Statement which in not clear how this fit with your results. Author response: There may be a relationship between low diatom abundance and high nauplii production without the relationship being causal, i.e., direct negative effects caused by diatoms on nauplii production. In principle it is possible that the end of the diatom bloom and copepod reproduction / peak abundance could have coincided. This has been explained in the manuscript.

Comment 9, Referee #1, L. 18-20, Please make clear that besides *E. affinis* nauplii you

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didn't count also nauplii from other species. Author response: We have clarified that only *E. affinis* nauplii were counted, P. 17099 L. 28 "Only first stage nauplii of *E. affinis* were included in the analyses".

Comment 10, Referee #1, L. 24-25, How this result is justified from your measurement? Do you have an approximate age for the recently matured adults? As age influences fecundity success it might be appropriate to put an approximate age of maturity to the individuals exposed. Author response: The development from hatching to adult for *E. affinis* females is estimated to be 17-24 days at 10-15 °C (Devreker et al., 2007, Seine Estuary) and the lifespan of the females can be up to 2 months (Devreker et al. 2012). We estimated the approximate age of the *E. affinis* adults incubated in our experiments to have been around 2-3 weeks old (newly matured) to >1 month.

Comment 11, Referee #1, L. 26-28, PUFA of which (females or eggs), please clarify? It is better to remove this to the next paragraph. P.17107 L.20-28. Author response: PUFA of females, this has now been included on L. 27. We considered moving the sentence to the next chapter (4.3) as suggested, but we decided to keep it on the original page as it is part of the discussion focussing on factors explaining the lower nauplii production towards the end of the experiment.

Comment 12, Referee #1, P. 17107 L. 20-28, Remove this paragraph to the previous chapter or modify it to attach better with this one. Author response: The paragraph has been moved to the previous chapter.

Comment 13, Referee #1, P. 17108 L. 19, "The possible pH stress *E. affinis* experienced in this study was rather via food. We found that the effects of food quantity had an impact on nauplii production of *E. affinis*. For the time we conducted the laboratory based experiments, we did not observe an indirect CO<sub>2</sub> effect via phytoplankton biomass". It sounds as a contradictory conclusion. The indirect effect is not very well described and discussed according to the results of this experiment. Author response: As pointed out by the referee the indirect effect is not extensively discussed. In this

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study we did not see an obvious effect of CO<sub>2</sub> on Chl a or phytoplankton during the experimental period (t3-t27). However, as also pointed out in the discussion (P. 17103 L. 21-24), a significant effect of CO<sub>2</sub> on Chl a was discernible after t25 onwards (result from Paul et al., this issue), but we only sampled until t27, so the possible indirect effects remains unclear. Sentence will be reformulated.

Comment: 14, Referee #1, Figure 1. Please, add the standard deviations in the plot. Figure 2. Add the trend line equation as well as r<sup>2</sup> and P values. Figure 3. Add the trend line equation as well as r<sup>2</sup> and P values. Figure 4. Add the trend line equation as well as r<sup>2</sup> and P values  
Author response: 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. Figure 2-4 Figure 2 presents the relationship between daily nauplii production and Chl a or diatom concentration. The graph includes repeated measures of the same group of individuals over four days, and repeated measurements of the same mesocosms over four weeks. Therefore, we used linear mixed effects models (LMM) with random structure taking into account these dependencies for analysing the dataset. We therefore cannot add correlation nor linear regression results to the figure legends. The statistical results corresponding to the figures are reported in Table 3. The same applies for Figures 3 and 4 (weekly, repeated measures of female fatty acid levels from the same mesocosms and weekly averages of female nauplii production, analysed with LMM).

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