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Comment

Interactive comment on “Copepod feeding and reproduction in relation to phytoplankton development during the PeECE III mesocosm experiment” by Y. Carotenuto et al.

Y. Carotenuto et al.

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Reply to Anonymous Referee #2 Comments.

RC 3915/11: I do not understand that a rate can be lower than a concentration.

AC: The cited sentence has been taken from Riebesell 2004 (Effects of CO₂ enrichment on marine phytoplankton, *Journal of Oceanography*, 60: 719-729). We will add this reference to the list.

RC 3916/16: I see this as an essential flaw of the study, as there is no replication in the CO₂ treatments. AC: The decision to monitor only one replicate bag for each pCO₂ treatment was taken in order to have a high female number per treatment, and, thus,

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a better statistical representation of the copepod population. It was also adopted by other studies of this issue (see Larsen et al. and Egge et al., which only monitored the M2 and M8 enclosures). Sure, the lack of replication could lead to a wrong interpretation of the potential CO₂ effects. However, after changing the statistic analysis of the data (as suggested by the referee later), we found that the copepod reproduction in the two pCO₂ levels only differed during the peak of the phytoplankton bloom. But more important, we also found similar results as those reported by the other studies, in regard to the phytoplankton development in M2 and M8. For example, we found that the total Chl a developing at the peak of the bloom did not differ significantly between M2 and M8, as also reported by Schulz et al. monitoring all replicate enclosures. Similarly, we observed that the abundance of some algal taxa (for example *E. huxleyi*, and nanoplankton) differed during the peak of the bloom, as reported by Paulino et al., this issue, monitoring all replicate enclosures. Since M2 and M8 enclosures, therefore, followed the general pattern of the other replicate enclosures, in regard to the phytoplankton dynamic, we believe that our interpretation of the potential CO₂ effect on copepods could also be representative of a CO₂-related effect on copepods, even though we have no copepod replication among the three replicates of each CO₂ treatment.

RC 3918/26: Why was reproduction monitored for such a long period? …any comparison between young and old females will confound age with food effects. AC: We monitored the copepod reproduction for the whole duration of the mesocosm experiment. However, the main difference in copepod reproduction between the two mesocosms was detected early in the experiment (days 8-10). It is, therefore, unlikely that the female age have played a role at this time of the experiment.

RC 3920/11: I would have thought that it was common knowledge that by taking the colour combination used in the figures a substantial part of the readership will not be able to differentiate between the lines. AC: You are right, we apologize for not having

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considered this. However, being our manuscript part of the special issue on the PeECE experiment, it was agreed to use these colour combinations.

RC 3921/1: This is certainly of interest, but we have no idea based on the data presented in this paper whether these differences have any significance. Due to the fact that there is no replication, these differences could be pure coincidental. AC: We have changed this part of the results in the revised version of the manuscript, following suggestions of Referee #1 and #2. The abundance of Nano1 over the experiment was significantly higher and less stable in the 3x CO₂ (M2) than in the present bag (M8) (Fig. 2, C). In particular, a significant higher Nano1 cell density developed in the M2 compared to the M8 enclosure during the peak of the bloom (days 7-13) (5.9 x 10³ cells ml⁻¹ and 3.9 x 10³ cells ml⁻¹, respectively) (Unpaired t-test, $t_6 = 2.697$, $p < 0.05$); Paulino et al. (this issue) monitoring all nine enclosures have also reported such a higher nanoeukaryotes concentration during the peak of the bloom.

By the way, the difference between the average abundances of Nano1 over the experiment in M2 (3x present CO₂) and M8 (1x present CO₂), we mentioned in the first version of the manuscript, was statistically significant (unpaired t-test $t = 2.549$, $df = 34$, $p < 0.05$). And this difference over the whole experiment is also statistically significant when all three bags for each treatment are analysed (3.3 x 10³ cells ml⁻¹ and 4.3 x 10³ cells ml⁻¹, for 1x and 3x, respectively; unpaired t-test $t = 4.213$, $df = 34$, $p < 0.001$).

RC 3922/5: I have strong objections against using a paired t-test for the statistical analysis in the experiment;..the values over the different days are not independent. AC: The referee is right. We have followed his/her suggestion and removed the paired t-test from the manuscript. To compare the two enclosures, therefore, we performed a classical unpaired t-test on average data over the entire experiment or over a chosen time-window (e.g. the peak of the phytoplankton bloom), when the copepod performance appeared to differ more.

RC: I do not understand the number of degree of freedom. AC: In order to compare

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average values for egg production, faecal pellets production, hatching success and recruitment rates in the two treatments, we calculated the total averaged produced eggs (or pellets, hatching and nauplii) per female per day during the complete experiment (ś individual variations). However, for a better and easier understanding of the results, in the revised version of the manuscript, we substituted these averages with the classical average over the entire experiment.

RC 3923/14: Again the statistical analysis of the data is incorrect. First of all there is no value for n in tables 1 and 2, but I assume the measurements through the time course have entered the analysis. There are several flaws in this analysis. Time course data are obviously not independent, algal densities on day 1 and those on day 2 will be fairly strongly correlated, because the algae cannot simply disappear or emerge. This means that by using all of the data in this analysis n gets artificially inflated, and correlations artificially increased as essentially the same comparison is made for a number of days....This makes the correlation analysis invalid and should be removed. AC: We have removed the correlation analysis from the revised version of the manuscript.

RC 3924/8: It is unclear, whether the data presented here are different from the ones presented in the Schulz et al paper. I would certainly hope that the data are in accordance with the ones in the Schulz paper, they are the same mesocosms. AR: Of course they are the same data. The meaning of the sentence was that the similarity and the differences we found in the phytoplankton bloom development between the two bags we monitored (M2 and M8), was also found by those studies that monitored the phytoplankton in all 9 enclosures. In order to make the sentence clearer, we will change it in the revised version of the manuscript. “This is in accordance with the reported development of the phytoplankton bloom when all the 9 mesocosms of the PeECE III experiment were monitored (Schulz et al., this issue, Paulino et al., this issue).”
RC 3926/13: I am not sure why this was not done, a direct C:N measurement would have helped, the drawdown is only indirect. AR: Our sentence was referring to the fact that no chemical analysis was performed in terms of cellular N or P, fatty acids, sterols,

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or toxic metabolites content of the algae. Instead, POC and PON accumulating in the mesocosms were measured. We have changed the discussion of the revised version of the manuscript in relation to this topic.

RC 3927/24: The conclusion is rather shaky, I am not sure which differences between treatments will remain when the data are analysed properly. Furthermore, I am not sure what the sentence However...;. Means. In the worst case it means that the authors selected the two mesocosms with the largest difference in dynamics, with others showing much less consistent patterns. AC: After following the referee's suggestions on the proper statistical analysis, we have changed the result and discussion sections. However, we decided to keep this sentence also in the revised version of the manuscript. With it, in fact, we meant to stress two aspects of the results. 1) The similarities and the differences we reported during the peak of the bloom between mesocosms M2 and M8, was also observed by others when all nine enclosures were monitored (see Paulino et al., and Schulz et al.). 2) These differences were very limited, in the sense that they were restricted to the time window of the peak of the bloom, and therefore, we cannot state that increased pCO₂ results in reduced copepod recruitment rates for the entire period;. In order to make the message clearer, we have slightly changed this sentence. However, because only very limited CO₂-related effects (e.g. restricted to the peak of the bloom), were observed on total standing stocks, taxonomic diversity and productivity of the primary producers when all replicates bags were also considered through out the entire PeECE III experiment (see other works in this volume), we cannot state that increased pCO₂ results in reduced copepod recruitment rates; for the entire period. ;

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