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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 #1 Comments. Interactive comment on “Copepod feeding and reproduction in relation to phytoplankton development during the PeECE III mesocosm experiment”;

RC: In principle, two types of CO<sub>2</sub>-induced effects may occur in herbivorous zooplankton with this experimental set-up: (1) direct effects of elevated pCO<sub>2</sub> levels and lowered seawater pH, e.g. on egg and larval development, and (2) indirect effects of e.g. altered food quality and quantity in response to differences in CO<sub>2</sub> enrichment. While the focus of this study clearly is on possible indirect CO<sub>2</sub> effects via changes in food quality, it is not entirely clear whether direct effects of pCO<sub>2</sub> and pH in the incubation

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medium can be completely excluded. Unfortunately, information about pCO<sub>2</sub> and pH in the medium used in incubations of both adult copepods and copepod eggs is missing in the manuscript.

AC: We agree with the referee that a CO<sub>2</sub>-related effect on copepods may involve a direct effect due to high pCO<sub>2</sub> and low pH, and an indirect effect due to a change in the food quantity/quality. As pointed out by the referee itself, and as clearly stated in the introduction of our manuscript, however, the aim of our work was to test the indirect effect of the increasing pCO<sub>2</sub> on the copepod secondary production. We did not perform, therefore, any measurements of the pCO<sub>2</sub> and pH at the beginning of the incubation and after the 48 hours period. We believe, however, that our results exclude any influence of the direct effect of the elevated CO<sub>2</sub> and low pH. We will discuss this in the revised version of the manuscript.

Methodological comments/questions: 1) The water used in the incubation experiments of the adult copepods was sampled daily from two of the PeECE III mesocosms (M2 and M8) and should therefore initially have the pCO<sub>2</sub> and pH levels occurring in the mesocosm upper mixed layer at the time of sampling. It is unclear, however, to what extent the sampling and handling of this water contributed to CO<sub>2</sub> gas exchange. Also, how did pCO<sub>2</sub> and pH change during the course of the 24 hour incubation? AC: We understand the referee concern about the possible effect of sampling, handling and incubation time on the CO<sub>2</sub> gas exchange. We guess his/her concerns are for the samples taken from the 3x mesocosm, which has the highest CO<sub>2</sub> concentration, and not the 1x treatment, that has the same CO<sub>2</sub> level as the present atmosphere. Unfortunately, we cannot quantify such exchange and its effect on the copepod performance, but if there were any influence of the high pCO<sub>2</sub> and low pH, this would have been higher at the beginning of the incubation. Since we did not maintain the 3x pCO<sub>2</sub> level in the incubation chambers, in fact, we would expect that, due to gas exchange, the CO<sub>2</sub> content of the water would decrease and the pH would increase during the 24-48 hours of incubation. Although we did not measure the CO<sub>2</sub> and pH change during

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the incubation period, we have the reference values measured in the mesocosm upper mixed layer at the time of sampling (Bellerby et al. 2007, this issue). According to Bellerby et al., the 3x present mesocosm had the highest pCO<sub>2</sub> content and the lowest pH at the beginning of the experiment (pCO<sub>2</sub> = 1050  $\mu$ atm, and pH = 7.64. As far as we are concerned, according to the published literature, such a pCO<sub>2</sub> and pH did not reduce copepod reproduction and egg viability. The two studies we are aware of, in fact, showed reduced egg viability at pCO<sub>2</sub> >8000 ppm and pH value well below 7.3 (Kurihara et al., 2004, Mayor et al. 2007).

RC 2. Was the same water used in female incubations also utilized in the egg hatching experiments? AC: Yes, the water used in female incubations was the same utilized for the egg hatching incubation experiment. If so, how did pCO<sub>2</sub> and pH levels change during the 48 hours of incubations? AC: See reply to point 1.

RC 3. Copepod ingestion rates were calculated from faecal pellet production measured in female incubations. The formula used to calculate ingestion rate is not clear to me: i) the numbers given in equation (1) do not appear to match with the units for ingestion rate and faecal pellet volume; ii) shouldn't the units for faecal volume read  $\mu\text{m}^3 \text{ f}^{-1} \text{ d}^{-1}$ , i.e. without the C? AC: Perfectly right. We did a mistake and wrote  $\mu\text{m}^3 \text{ C f}^{-1} \text{ d}^{-1}$  instead of  $\mu\text{m}^3 \text{ f}^{-1} \text{ d}^{-1}$ . We will correct it.

RC 4. P. 3916, lines 16-17: Initial pCO<sub>2</sub> levels in the 1x and 3x CO<sub>2</sub> treatments were 350 and 1050  $\mu$ atm, respectively. AC: Right. We apologize for the mistake. We will correct it.

RC 5. P. 3919, line 17: There was no silicate added to the mesocosms. AC: Right. We will correct it.

RC 6. P. 3925, lines 8-9: POC accumulation should not be regarded a sufficient indicator for effects at the phytoplankton community level. AC: Right. We will rephrase the sentence in &#8220;&#8230;showed that although the POC accumulation remained unaffected at a pCO<sub>2</sub> of 750 ppm, the diatom *Skeletonema costatum*..&#8221;.

Comments on data presentation and interpretation: RC 1. P. 3915, lines 12-13: I think it's not so clear what is the predominant carbon source used for photosynthesis by *Emiliana huxleyi*. AC: The referee is sight. We will delete the sentence and mainly relies on dissolved CO<sub>2</sub> concentration for photosynthesis;

RC 2. P. 3915, lines 24-26: This is a model study relying on multiple (and partly untested) assumptions. Hence, a predicted 14% contribution of copepod grazing to calcite dissolution should be seen as a working hypothesis rather than as evidence for copepod induced calcite dissolution. AC: We will rephrase the sentence in this one: In addition, copepod feeding on calcifying organisms may also have implications for carbonate dissolution. A numerical model, in fact, predicted that in pre- or post-bloom situations copepod grazing might give rise to 14% of calcite standing stock dissolved in copepod guts. (Jansen and 25 Wolf-Gladrow, 2001).

RC 3. P. 3920, para 1: The apparent difference in Chlorophyll a between mesocosms 2 and 8 is not representative for the respective CO<sub>2</sub> treatments when considering the triplicate mesocosms (see Schulz et al., this volume). The way it is stated here may give a false impression regarding possible effects of CO<sub>2</sub> on phytoplankton development. AC: We will change this part in the revised version of the manuscript according to the modified statistical analysis suggested by Referee #2. According to this re-analysis, in fact, the difference between M2 and M8 was not statistically significant any more.

RC 4. P. 3920, lines 11-12: "... with up to 5.6  $\mu\text{g l}^{-1}$  in both mesocosms ..." Referring to diatom or prymnesiophyte Chl. a? AC: We will specify it in a corrected sentence. In both mesocosms, pigments based chemotaxonomy showed dominant contributions of diatoms and prymnesiophyceae (mainly *Emiliana huxleyi*) to total Chl a in both M2 and M8 (Fig. 1), with up to 6.5  $\mu\text{g l}^{-1}$  and 5.6  $\mu\text{g l}^{-1}$ , respectively, at the peak of the bloom.

RC 5. Section 3.2 Phytoplankton development and Figs. 1 and 2: Much of the infor-

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mation provided in this section and in the corresponding figures (based here on only 2 mesocosms) is presented in a more comprehensive way for all 9 mesocosms in the manuscripts by Schulz et al. and Paulino et al. (both in this volume). The description here should therefore focus on differences immediately relevant to the zooplankton experiments reported in this study. Also, restricting a data comparison to only two mesocosms may give a wrong impression regarding potential CO<sub>2</sub> treatment differences. AC: We agree with the referee that a data comparison restricted to only two mesocosms may give a wrong impression of any CO<sub>2</sub> effect on copepods, but since our copepods were only feeding on the water coming from M2 and M8, we believe it is more appropriate to compare only these two enclosures. However, as suggested by the referee, in the revised version of the manuscript, we will restrict the data description to those differences more relevant to the copepod performance.

RC 6. P. 3925, bottom line - p. 3926, first line: "This may be explained by a combination of food saturation and/or lower quality/deleterious food composition in the 3x CO<sub>2</sub> treatment." It is not clear what the statement in italics is based upon. The POM data presented in Schulz et al. (this volume) do not show significant treatment differences. AC: This statement is reasoning on certain possible causes that might have induced the same egg production but different recruitment of the copepods in the two CO<sub>2</sub> treatments. Later in the same page (line 1-8), then, we found evidence that the copepods were food saturated at already 1x CO<sub>2</sub>, and therefore the egg production was not improved at 3x CO<sub>2</sub>. We thought we had also find evidence that the lower recruitment at 3x CO<sub>2</sub> was due to the higher C:N content of the planktonic community, according to Riebesell et al. 2007, which reported a higher DIC:N drawdown at 3x CO<sub>2</sub>. However, since the drawdown is an indirect measurement of the C and N content of the POM, and, more importantly, the manuscript by Schulz et al (this volume) did not find any difference in the POC:PON between CO<sub>2</sub> treatments (as pointed out by the referee), we will smooth this part of the discussion in the revised version of the manuscript, making clear that this is a speculative hypothesis on what may have affected the lower copepod recruitment at the 3x CO<sub>2</sub>.

Pag 3925 bottom line-3926 first line will be changed into: This could be explained by a combination of food saturation and/or lower quality/deleterious food composition in the 3CE CO2 treatment.

RC 7. P. 3926, lines 8-13: Is there any evidence (in the literature or from this study) indicating a connection between CO2 and any of the proposed causes for recruitment efficiency? AC: Yes. There is in the literature some evidence of changes in the phytoplankton cellular C:N:P composition in response to increasing pCO2 (some of these articles are also suggested by the referee itself in the subsequent point 9). We will add them in the revised version of the manuscript together with a short paragraph. Pag. 3926, line 12: It has been shown, in particular, that the phytoplankton cellular C:N:P composition may change in response to increasing pCO2, showing large species-specific differences among taxa (Burkhardt et al. 1999). Although Burkhardt and co-workers did not observed a clear trend in the response (either an increase or a decrease with increasing the CO2 concentration), and the pCO2 at which the algae were sensitive was below the present CO2 level, we should take into account that these experiments were conducted under nutrient replete and high light conditions, and hence when weak deviations from Redfield ratios are expected. A recent study using nutrient and light conditions typically occurring in the natural lake environment, for example, showed that increased pCO2 reduced the algal P:C ratio and, in turn, reduced the growth of the freshwater planktonic herbivore *Daphnia pulicaria* (Urabe et al. 2003).

RC 8. P. 3926, lines 18-19: Note that the enhanced DIC relative to nitrate drawdown in response to elevated CO2 is not mirrored by a corresponding signal in the C:N of suspended organic matter (see Schulz et al. (this volume) for POM stoichiometry and Riebesell et al. (2007) for a mechanism possibly explaining this discrepancy). AC: See our reply to point 6. The sentence will be changed in the revised version of the manuscript in: while the lower nauplii recruitment during the top of the bloom in the 3x mesocosm could be due to a high and unfavourable stoichiometric

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carbon to nitrogen (C:N) content in the plankton community.&#8221;

Pag. 3927, line 17: &#8220;However, whether slightly elevated C:N ratios are negative for copepod reproduction success have been debated (Augustin and Boersma, 2006). In addition, the higher DIC relative to nitrate drawdown was not mirrored by a corresponding higher POC:PON accumulation in the surface layer, which, in fact, was unaffected by the CO<sub>2</sub> treatment and remained close to Redfield ratio (Riebsell et al., 2007, Schulz et al., 2007, this volume). Consequently, the question on whether the (inorganic) nutritional level of the 3x pCO<sub>2</sub> treatment affected the copepod hatching success and naupliar recruitment is still open. The net impact on copepods from increased future CO<sub>2</sub> levels, therefore, may be very complex, and future manipulative experiment should takes into account the biochemical and elemental cellular composition of the phytoplankton too.&#8221;

9. P. 3927, lines 17-23: See also the following references: Urabe et al. (2003) *Global Change Biology* 9, 818; Jeyasingh (2007) *Ecology Letters* 10, 282-289; Jensen & Hessen (2007) *Oecologia* 152, 191-200. AC: Many thanks. We will add the reference Urabe et al. 2003 to the revised version of the manuscript. However, because the references Jensen & Hessen (2007) and Jeyasingh (2007) are on the mechanisms used by the freshwater *Daphnia* to cope with the higher carbon uptake due to a high C:P diet (e.g. higher respiration), we feel that they do not fit properly to the discussion of our results. We therefore decided not to add them in the manuscript.

RC 10. P. 3927, lines 24-26: see points 6 and 8 above. AC: See also our reply to the corresponding points. This part will be changed in the revised version of the manuscript: &#8220;In conclusion, one may speculate that the higher algal biomass (dominated by diatoms, *E. huxleyi*, and nanoplankton) developing during the peak of the bloom in the 3x CO<sub>2</sub> environment, could have been inferior food for *C. finmarchicus* hatching success and naupliar recruitment, compared to the prey field in the present (1x) CO<sub>2</sub> environment.&#8221;

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Editorial comments:

RC 1. P. 3915, lines 3-8: The list of impacts of rising atmospheric CO<sub>2</sub> given in this paragraph mixes impacts which will occur with absolute certainty (e.g. altering buffer capacity and changing seawater carbonate chemistry) with others, which are more speculative (changing the strength of the biological pump). AC: The sentence will be rephrased: "The rising of atmospheric CO<sub>2</sub> could greatly impact the ocean food webs and the global carbon cycle, altering the buffering capacity (pH) and the carbonate chemistry of seawater, with important consequences for organisms with calcareous skeletons as coccolithophorids, corals and molluscs. Such modifications may also change the strength of the biological pump, which drives the carbon export from upper to deep oceans via carbon fixation by photosynthetic organisms";

RC 2. P. 3922, lines 3-4: Shouldn't it read "... both..., and ..." instead of "... either ..., or ..."? AC: Right. We will correct it.

RC 3. P. 3924, line 11: delete reference to Schulz et al. since the paper does not provide data relevant to this statement. AC: Right. We will delete it.

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