

Interactive comment on “Chronic exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO₂-acidified seawater; effects on survival, growth and development” by S. A. Pedersen et al.

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We wish to thank referee #2 for a critical and constructive review and insightful suggestions for modifications and additions to improve our paper.

Referee #2: Introduction: There are quite a lot of grey literature references in the introduction, one of which is a literature review (Turley 2004). Could any of them be replaced by relevant peer review publications? Response: We suggest replacing Turley 2004 with a work by Gerlagh and van der Zwaan 2011 on Page 5275 line 21: “Sub-

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seabed storage is considered to be a relatively safe method to dispose of CO₂, but risk assessments suggest that a leak may occur within the next 1000 yr, through processes such as cap rock failure, where up to 2% of the stored CO₂ could be lost to the water column (Turley et al., 2004).” → “Sub-seabed storage is considered to be a relatively safe method to dispose of CO₂, but risk assessments indicate that loss of stored CO₂ to the water column could occur through leakage (Gerlagh and van der Zwaan 2012).

Referee #2: Page 5276, line19: It seems odd to choose Shek and Liu 2010 as a single reference to highlight the role that copepods play in marine food webs as it is only concerned with one aspect of their importance, i.e. faecal pellets. Could you add more references, e.g. concerned with their grazing, importance as fish food, or a more inclusive reference. Response: We agree, and suggest that the Shek and Liu 2010 is replaced by a more general reference used previously in the manuscript on Page 5276, line 19: “Copepods are small crustaceans that play a vital role in marine food webs (Shek and Liu, 2010).” → “Copepodes (Crustacea; Copepoda) are considered to constitute the most numerous multicellular organisms on Earth (Mauchline, 1998), and thus play a vital role in marine food webs.”

Referee #2: Page 5276, line 25: You state that Kurihara & Ishimatsu 2008 found negative effects of CO₂ on *Acartia tsuensis* but they found no significant effects. Their text states that egg hatching was significantly lower overall in the CO₂ incubations but this significant difference did not hold when the different generations were considered separately. Response: Kurihara and Ishimatsu 2008 write the following in the results section: “The overall hatching rate of CO₂ eggs was significantly lower than that of the control eggs (Fig. 5, $P < 0.05$, two-way ANOVA) however there was no significant difference in the hatching rate between the control and CO₂ eggs when compared separately for each generation (Fig. 5, t-test).” We mean that the overall effect observed on hatching success is important to consider, since it integrates the effect of CO₂ over the three generations. We suggest the following change to incorporate the point of the referee on Page 5276, line 25: “While some species seem to tolerate CO₂ levels

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that are well above 2000 ppm (the level expected for year 2300), others such as *Acartia* *tsuensis* (Ito) (Kurihara and Ishimatsu, 2008), have been showed to be negatively affected at CO₂ levels \geq 2300 ppm." \rightarrow "While some species seem to tolerate CO₂ levels that are well above 2000 ppm (the level expected for year 2300), others such as *Acartia* *tsuensis* (Ito) (Kurihara and Ishimatsu, 2008), displayed an overall reduced hatching success in the eggs produced at 2300 ppm CO₂ when incubated over multiple generations (although no significant difference was observed within each separate generation)."

Referee #2: Page 5278, line 10: You state that Zhang et al 2011 found no effect on egg production rate of *Calanus sinicus* up to 10000ppm CO₂, however there were negative effects at 10000ppm. Did you meant no effects at less than 10000ppm? Response: Yes, we suggest the following correction on Page 5278, line 10: "In wild-caught *Calanus sinicus* (Brodsky), no effect on adult survival and egg production rate was observed during an eight day long incubation period in seawater with a CO₂ level of up to 10 000 ppm (Zhang et al., 2011)." \rightarrow "In wild-caught *Calanus sinicus* (Brodsky), no effect on adult survival and egg production rate was observed below 10 000 ppm CO₂ during an eight day long incubation period (Zhang et al., 2011).

Referee #2: Methods: Page 5279, line 1: I don't understand what is meant by 'mature water'. Response: Referee #1 has also raised the same question. To avoid unnecessary confusion we suggest that this term is removed from the sentence. We propose that the following modification on Page 5279 line 26 (-P 5279 L1); "which include using a combination of heavy aeration and sprinkling over biofilm carriers (Kaldnes Miljøteknologi, Norway) in polyethylene holding tank (6m3), to obtain mature water." \rightarrow "which include using a combination of heavy aeration and sprinkling over biofilm carriers (Kaldnes Miljøteknologi, Norway) in polyethylene holding tank (6m3)."

Referee #2: Page 5279, line 8: How many females were incubated in order to collect eggs? Response: The eggs were collected from 240 females. We suggest the following change to incorporate this information on MS on page 5279, line 8: "The females

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were transferred to a 50 L polyethylene tank.." \rightarrow "The females (240 individuals) were transferred to a 50 L polyethylene tank..".

Referee #2: Page 5281, line 7: Was the 'stable density of algae' measured or checked for stability? Is this combination of algae the same as is fed to the culture animals? Response: Yes, the algae was checked for stability and found to be high throughout the experiment and also towards the end when the appetite of the animals was at the maximum. Yes, the combination of algae that was used for the experiment was the same as that used for the culture animals.

Referee #2: Page 5282, line 16: Did you do any checks to see how stable the pH/ carbonate chemistry were, and if weekly checks were sufficient? Response: The stability of the system with respect to pH/ carbonate chemistry was checked prior to the experiment. The test confirmed that the system was stable enough for weekly measurements to be sufficient during the experiment.

Referee #2: Results: Page 5284, line 3 (and Figure 4): It would be useful to know how many of each stage were measured. Response: We suggest that the following information is added to the legend in Figure 4. on page 5300: "Numbers of individuals measured were: CIII = 43, CIV = 254, CV = 610, CVI = 22." Referee #2: Discussion: Page 5285, line25: You state that 2000ppm is the worst case scenario predicted for the year 2300 but don't add a reference (Caldeira & Wickett 2003?). Caldeira & Wickett 2005 use 8000ppm as a worst case scenario. Response: The work by Caldeira & Wickett (2003) should have been cited. We suggest the following modification on Page 5285, line25: "Collectively, the results so far available on *Calanus* species suggest that CO₂ levels \leq 2000 ppm (the worst case CO₂ level predicted for the year 2300) is not likely to directly affect the survival of individuals from this genus." \rightarrow "Collectively, the results so far available on *Calanus* species suggest that CO₂ levels \leq 2000 ppm (the worst case CO₂ level predicted for the year 2300 by Caldeira and Wickett (2003)) is not likely to directly affect the survival of individuals from this genus."

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Referee #2: Page 5286, line 2: You give Pascal et al 2010 as a reference for adult copepods being more resistant to elevated CO₂ than eggs and nauplii, however that study only looked at nonovigerous females. Response: Pascal et al. 2010 is clearly not appropriate in this context. We suggest that this citation is removed from Page 5286, line 2: "Indeed, adult copepods have been found to be much more resistant to elevated levels of pCO₂ than eggs and nauplii (Kurihara et al., 2004; Pascal et al., 2010; Mayor et al., 2007)." → "Indeed, adult copepods have been found to be much more resistant to elevated levels of pCO₂ than eggs and nauplii (Kurihara et al., 2004; Mayor et al., 2007)."

Referee #2: Page 5286, line 13: The Kurihara et al 2004 reference that is relevant here is not listed in the reference section (Kurihara, H. et al. Effects of raised CO₂ concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*). *Marine Pollution Bulletin* 49, 721-727, 2004. Response: The referee is correct. The work will be included in the reference list on Page 5292 line 24: "Kurihara, H., Shimode, S. and Shirayama., Y.: Effects of raised CO₂ concentration on the egg production rate and early development of two marine copepods (*Acartia steueri* and *Acartia erythraea*), *Mar. Pollut. Bull.* 49, 721-727, 2004."

Referee #2: Page 5286, line 29: You have only listed one species/reference for effects of CO₂ on fertilisation when there are several others in the literature. Listing a couple more would add weight to the argument. Response: We agree to the suggestion by the referee and propose to add two additional works on Page 5286, line 29: "Indeed, near future CO₂ levels have been found to affect fertilization processes in other invertebrate species, including the sea urchin *Heliocidaris erythrogramma* Valenciennes (Havenhand et al., 2008)." → "Indeed, near future CO₂ levels have been found to affect fertilization processes in other invertebrate species, including the sea urchin *Heliocidaris erythrogramma* Valenciennes (Havenhand et al., 2008), the oyster the oyster *Crassostrea gigas* (Barros et al. 2013) and the Antarctic seastar *Odontaster validus*

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(Gonzalez-Bernat et al. 2013)."

Referee #2: Page 5287, line 1: You again state that Kurihara & Ishimatsu 2008 found negative effects of CO₂ on *Acartia tsuensis* survival but they found no significant effects. Response: We suggest that the following sentence is added to clarify on Page 5287, line 1: "In the study by Kurihara and Ishimatsu (2008), an overall reduction in the hatching success was observed when the results from three consecutive generations exposed at 2300 ppm CO₂ were compared to the control, but no significant effect was observed within the separate generations."

Referee #2: Page 5287, line 13: The reference should be Kurihara & Ishimatsu 2008, not Kurihara et al 2008. Response: We will replace the citation on Page 5287, line 13: "to 2300 ppm CO₂ in a multiple generation study on *A. tsuensis* (Kurihara et al., 2008)." → "to 2300 ppm CO₂ in a multiple generation study on *A. tsuensis* (Kurihara and Ishimatsu 2008)."

Referee #2: Tables & Figures: Table 1: This table needs units. We suggest the following correction: "AT S T pCO₂ CT °C Ca Ar" → "AT (µmol/kg SW) S (PSU) T (°C) pCO₂ (ppm) CT (µmol/kg SW) °C Ca Ar"

Referee #2: Technical corrections: Species names have the taxonomic authorities but not the dates. Response: We will add dates where taxonomic authorities are listed.

Referee #2: Fat content of animals is referred to throughout the manuscript, but lipid content may be a better term. Response: We agree, and will change the term "fat" to "lipid" throughout the text.

Referee #2: Page 5274, line 20: Suggest change 'raise' to 'a rise in'. Page 5275, line 1: Change 'middle' to 'mean' or average? Page 5275, line 5: Is 'A1F' supposed to be there? Page 5275, line 7: Suggest change 'Even up to 8000ppm has been ' to '8000ppm has even been'. Page 5276, line 1: Suggest change 'raised on' to 'regarding'. Page 5276, line 28: Change 'revealed negative' to 'revealed a negative'.

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Page 5277, line 1: Suggest change the word 'nuancing' to 'contradicting'. Page 5277, line 29: Suggest change 'at the same time as' to 'although'. Page 5279, line 22: Define ID on first use. Page 5280, line 6: Define AGA on first use. Page 5280, line 13: Define OD on first use Page 5280, lines 20 & 21: Change 'basis' to 'base'. Page 5281, line 1: Change 'feed' to 'fed'. Page 5283, line 17: Suggest remove the word 'single'. Page 5284, line 27: Suggest change 'of what' to 'that'. Page 5285, line 3: Suggest change 'is likely to' 'may' as it is only animals overwintering in CCS areas and only in case of a leak that may be affected. Page 5285, line 18: Suggest change 'of what' to 'that'. Page 5285, line 21: Change 'in light of this the' to 'in light of the'. Page 5285, line 29: Change 'retards' to 'regards'. Page 5286, line 8: Suggest change 'for the successful' to 'for successful'. Page 5287, line 26: Suggest change 'potentiated' to 'intensified'. Page 5288, line 17: Change 'Zooplanktons' to 'Zooplankters'. Page 5289, line 17: Suggest change 'reservations' to 'caution'. Table 1: S and T need to be defined. Fig 1: Change 'innlet' to 'inlet' and 'entiched' to 'enriched'. Fig. 4 Change 'lenght' to 'length' Response: We agree with the comments and will incorporate them into the manuscript.

References:

Gerlagh, R. and van der Zwaan, B.: Evaluating uncertain CO₂ abatement over the very long term, *Environ. Model. Assess.*, 17, 1–12, 2011.

Barros, P., Sobral, P., Range, P., Chicharo, L. and Matias, D.: Effects of sea-water acidification on fertilization and larval development of the oyster *Crassostrea gigas*, *J. Exp. Mar. Biol. Ecol.* 440, 200-206, 2013.

Gonzalez-Bernat, M. J., Lamare, M. and Barker, M.: Effects of reduced seawater pH on fertilisation, embryogenesis and larval development in the Antarctic seastar *Odontaster validus*, *Polar Biol.*, 36, 235-247, 2013.

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