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Interactive comment on "Chronic exposure of the North Atlantic copepod *Calanus finmarchicus* (Gunnerus, 1770) to CO_2-acidified seawater; effects on survival, growth and development" *by* S. A. Pedersen et al.

S. A. Pedersen et al.

sindre.pedersen@bio.ntnu.no

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We thank referee #1 for a critical and constructive review and insightful suggestions for modifications and additions to our paper.

Referee #1: "I have some concern (1) about the usefulness of the data obtained under those levels," "(1) Why did the authors select these levels? In page 5285, Vetter and Smith (2005) is cited to argue that the highest level used is relevant to estimate impact by CO2 leakage from a subsea site. But the paper does not say anything about

expected CO2 level due to leakage. How can these data contribute to risk assessment of CO2 leakage from a CCS site? The authors are encouraged to state more clearly rationale for their choice of CO2 levels." Response: The level of CO2 used in the present study was selected based on two main criteria. Our main priority was to include CO2 levels that were relevant to the conditions that might occur in relation to a leakage situation from a sub-seabed carbon storage site. Secondly, we wanted to include treatment levels that would show the sensitivity-range and help identify the physiological tipping point of Calanus. Based on acute tests with varying CO2 levels we suspected that Calanus might be robust with regards to the CO2 levels that are relevant to the near-future projections. Accordingly, a CO2 level approximately a 1000 ppm above the 2300 year worst case scenario for atmospheric CO2 was chosen for the lowest CO2 treatment. The title of the cited study by Vetter and Smith (2005) is "Insights into ecological effects of deep ocean CO2 enrichment: The impacts of natural CO2 venting at Loihi seamount on deep sea scavengers". Referee #1 raises a valid point since it is correct that this paper deals with a natural CO2 vent and does not state directly what levels may be expected in relation to a leakage event from a CCS-storage site. To improve we suggest that the study by Vetter and Smith (2005) is replaced in favor of a study by Blackford et al. (2008). Blackford et al. (2008) have modeled the potential effect of leakage on the seawater pH (a pH drop by up to one unit) and found that some of the leakage scenarios could produce pH changes in the range of a whole unit (e.g. a pH corresponding to the high CO2 treatments in our study). We suggest the following change on Page 5284 line 27- Page 5285: "Such a level of CO2-induced acidification is within the range of what may be relevant to episodes of leakage from sub seabed storage sites for CO2 (Vetter and Smith, 2005)." –>"Such a level of CO2-induced acidification is within the range of what may be relevant to episodes of leakage from sub seabed storage sites for CO2 (Blackford et al., 2008).

The data form our study show that Calanus might be affected by CO2-levels that may occur in the water in the vicinity of a leaking sub-sea bed carbon storage site. However, while we must take some reservations with regards to potential effects on the

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fertilization process (not examined in the present study) the absence of any apparent effect on hatching and survival at 3300 ppm CO2 (the lowest CO2-treatment) makes it unlikely that a CO2 level $\leq$ 2000 ppm (i.e. worst case scenario for the end of year 2300) alone will directly affect the hatching success or later survival in C. finmarchicus. In this respect we argue that the results from our study are highly relevant with respect to risk analysis for this species.

Referee #1: "(2) There are published studies on copepods that examined CO2 effects, and we know already something about their responses to acidified environment. Then, what is the new knowledge we can learn from this study? Is this not a repeating of what has already been done with just another species? Please stress any new insights generated by this study." Response: As we write in the introduction of the manuscript, copepods show highly variable sensitivity to CO2 acidification and this seems to be both stage and species specific. The Calanus complex is considered to be of particular interest due to the key role of these species in the marine food chains. The knowledge of their sensitivity to elevated CO2 is limited to a few studies where wild caught animals have been transferred to lab conditions and acutely (a few days) exposed to observe the effect on the production of eggs and their hatching success. Our study presents results from a laboratory study of CO2 acidification effects on Calanus finmarchicus over almost one complete life cycle (excluding reproduction), integrating the effects on growth, development and survival over all life stages. In our view this represents a significant improvement with respect to estimation of the more long term effects. The acute studies have indicated that exposure to 1000 ppm CO2 may not affect hatching success in Calanus species. However, these studies does not indicate if these species are tolerant to the worst case scenarios for year 2300 (2000 ppm) or higher CO2 levels. Our study shows that the survival was not significantly affected by exposure to 3300 ppm CO2. This information is interesting because it indicates that there may be a margin of safety by approximately a 2000 ppm compared to the worst case CO2 emission scenario for year 2100 (1000 ppm CO2). The other main finding is that the species displays strong negative effects in terms of survival, growth and de-

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velopment when exposed to CO2 levels that are be relevant to the conditions that may occur in a plume produced by a leakage from a sub-seabed carbon storage site.

Referee #1: ÂńSpecific comments Title: I think that the word “chronic” is not appropriate because it means “marked by long duration or by frequent recurrence over an extended period of time and often by slowly progressing seriousness : not acute”(Merriam-Webster Unabridged). The authors may use “medium-term exposure” or “28-day exposure” instead.Âż Response: The referee rises a valid point. We suggest that the title is changed to: “Medium-term exposure of the North Atlantic copepod Calanus finmarchicus (Gunnerus, 1770) to CO2-acidified seawater; effects on survival, growth and development”

Referee #1: Abstract: The use of “microcosm” is disturbing. The words “microcosm”, “mesocosm”and “macrocosm” are most often used when studying interactions of different species in an enclosure of different size. Why do not the authors call it just as an experimental setup/system? Response: The referee raises a valid point. The following change is suggested on Page 5279, line 13: “A custom flow-through microcosms system was developed. . . “ –> “A custom flow-through experimental system was developed. . .”.

Referee #1: Methods: In 5281 L12, it is stated “240 newly laid eggs were...transferred to each incubation chamber...”. And there were 12 chambers (4 CO2 levels x 3 replicates). This means that at least 2880 eggs were used. Were all the exposure experiments conducted on the same dates? Or there were some staggering? And from how many females were these eggs originated? Response: Yes, it is correctly observed that a total of 2880 eggs were used for all of the experimental units. To avoid problems with the picture taking (when the experiment was terminated), we used a staggering strategy, where each of the replicates for the four different experimental treatments (control, 3300, 7300, 9700 ppm CO2) was started with one day interval. This meant that one day one replicate one of control, 3300, 7300 and 9700 ppm CO2 was started, and so on. The number of pregnant females used to collect eggs for the experiment

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was 240. It is difficult to estimate how many of the 240 females that actually contributed to the egg production. An examination of the egg production on ten individuals showed that the egg production was on average 11 per female per day, and the number of eggs produced varied from 0 to 20. The number of females used to produce the eggs is also inquired by referee #2. To avoid uncertainties we suggest that the following modifications is made to the MS on Page 5279, line 8: "The females were transferred to a 50 L polyethylene tank.." –> "The females (240 individuals) were transferred to a 50 L polyethylene tank..".

Referee #1: Results: In 5282 L13 The authors stated "Copepodites stage and sex of adults were determined...". Were there adults after 28 days of exposure? If so, please state the number of males and females in each condition. Response: Yes, there were adults (denoted CVI in the MS) present after 28 days of exposure. The proportion of adults at the end of the experiment is depicted in figure 3. The animals only reached the adult stage in the control groups and in the groups that were exposed to 3300 ppm CO2. Regarding the number of males and females in each treatment group we found no males among the adults. This information is presented on p. 5284, L1-2 in the MS: "No males were present among all the CVI at day 28". Referee #2 also inquires information on the number of adults present at the end of the experiment. To improve this point we suggest that this information is added to the results section on Page 5284 line 1: "While adults (CVI) constituted 5.87 ($\pm$4.19) and 0.53 ($\pm$0.46) % of the animals at the end of the experiment in the control and 3300 ppm treatment, respectively, no adults developed in the two highest CO2 treatments (7300 and 9700 ppm)."

Referee #1: On page 5279 L1 What is "mature water"? Response: Referee #2 have also raised the same question as referee #1. To avoid unnecessary confusion we suggest that this term is removed from the sentence. We propose that the following modification on Page 5279 L26-5279 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." –> "which include using

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a combination of heavy aeration and sprinkling over biofilm carriers (Kaldnes Miljøteknologi, Norway) in polyethylene holding tank (6m3)."

Referee #1: 5279 L8 How many females did the authors use? Response: We used 240 females to produce the eggs. We have suggested including this information in the M&M section (see earlier comment)

Referee #1: 5279 L8 Did the authors used only one tank? In this way, it seems not possible to estimate eggs originated from how many females. Response: Only one tank was used to collect eggs from the 240 females. As explained above, we checked the egg production among the females before the experiment. The females produced on average 11 eggs per day, and although the number of eggs produced varied, the majority of the individuals produced some eggs each day. It is difficult to determine exactly how many of the 240 females that contributed to the egg production, but we think that it is reasonable to assume that most of them contributed.

Referee #1: 5280 L4-11 Did the authors measured CO2 concentrations of these gas mixtures? If not, how the authors confirmed? Response: The CO2 level in the different gas mixtures that were produced by the gas mixing system was determined using a NIR CO2 gas analyzer calibrated using CO2-free air and a 1% CO2 gas standard.

Referee #1: 5281 L1-10 Did the authors confirmed plankton concentrations? The food was always sufficient? Response: The plankton level in the different treatments was verified by collecting water samples from the outlet of the exposure bottles. The algal level was monitored and always found to be high. Even at the end of the experiment, when the appetite of the copepods are at their highest no noticeable change in algal level was apparent. Equilibrated seawater with added algae was added to the experimental units at a flow rate of 2.5 mL/min. This flow rate corresponded to a full water exchange two times per day in the bottles.

Referee #1: 5281 L12Was the exposure to all CO2 levels started on the same day? If so, the authors must have needed about 3000 eggs (240 x 12). For what did the authors

sort the eggs? Did the authors confirm the eggs were all fertilized? Response: The first part of the comment has been addressed above already (see earlier comment). The eggs were sorted to collect eggs that were as newly laid as possible. New eggs have a more transparent appearance than the older ones and this feature was used to secure that the eggs used in the experiment were as newly laid and as synchronized as possible. We did not confirm that all eggs were fertilized. The sorted eggs were randomly distributed between the different treatments to avoid potential bias.

Referee #1: 5281 L26-27 Without water renewal, there might have been accumulation of waste materials in the experimental seawater. Do the authors have data for ammonia etc in seawater? Response: The experimental system used was based on constant flow through that constitutes a change of the total volume of the exposure bottles twice a day. With the animal density that was used in the present experiment the risk of any ammonia buildup was considered to be minimal. Although the cleaning procedure involved a temporarily stop in the water flow, the whole procedure had a total duration of approximately one hour, and the animals were confined to a small volume for only a short period (minutes). With the modest bio-load in the bottles, and the brief period with stagnant water during the cleaning procedure, ammonia build up was not considered to pose a threat. Ammonia measurements were therefore not included in the experimental procedure.

Referee #1: 5282 L24 “derived species”? Response: “derived species” refers to the carbonate species that were calculated from pHTot, total alkalinity and salinity using the CO2SYS software.

Referee #1: 5283 L17 What are “two single nauplii”? Was there one nauplius or two nauplii? Response: The correct is “two nauplii”. On Page 5283 line 17 we suggest the following modification: “With the exception of two single nauplii found in one of the replicate bottles,” → “With the exception of two nauplii found in one of the replicate bottles,”

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Referee #1: 5284 L25 “suggest” > “suggests” Response: We will make the correction

Referee #1: 5286 L19-22 When the authors had already realized this, why then did not the authors employ an experimental protocol to reveal this? Response: We have chosen to give priority to determine the integrated effect of CO2 acidification on the growth, development and survival over all life stages by exposing Calanus finmarchicus over almost one complete life cycle. Determining the relative sensitivity of the different life stages, which may explain the integrated effects, observed in this study, will be top priority in our future experiments.

Referee #1: 5286 L23-5287 L5 Why did not the authors attempt to do this? Response: As we write in the MS, the experiment did not include CO2 exposure during the fertilization process. Successful mating in Calanus finmarchicus has proven to require relatively large holding tank. At the time of this study we lacked the infrastructure to accommodate this in our experimental protocol.

Referee #1: Table 1 Add units. Response: ” AT S T pCO2 CT âĎęCa âĎęAr” will be corrected to ” AT (μmol/kg SW) S (PSU) T (°C) pCO2 (ppm) CT (μmol/kg SW) âĎęCa âĎęAr”

Referee #1: Fig. 4 Add number of individuals. Response: We suggest that the following information is added to the legend in Figure 4: “Numbers of individuals measured were: CIII = 43, CIV = 254, CV = 610, CVI = 22.”

Referee #1: Technical corrections 5275 L1 “middle”? “mean”? Response: “middle” should be changed to “mean”.

Referee #1: 5275 L2 “is” > “has been” 5281. L1 “feed” > “fed” 5284 L16 “more short-term” > “shorter-term” 5284 L18 Delete “the survival in terms of low” 5285 L3 Delete “a” before CO2. 5285 L3 Delete “it”. 5285 L7“is” > “are”. 5285 L11 “within” > “by”. 5285 L12-13 Delete “survival, in terms of”. 5285 L29 “retards” > “regards” 5289 L8 “ration” > “ratio” Response: We agree with all the corrections suggested above and include them

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in the revised manuscript.

Referances: Blackford, J., Jones, N., Proctor, R., Holt, J., Widdicombe, S., Lowe, D., and Rees, A.: An initial assessment of the potential environmental impact of CO2 escape from marine carbon capture and storage systems, P. I. Mech. Eng. A, 223, 269-280, 2009.

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