

Interactive comment on “Ammonium excretion and oxygen respiration of tropical copepods and euphausiids exposed to oxygen minimum zone conditions” by R. Kiko et al.

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Dear Editor, Dear Referees, We would like to thank you for the helpful comments to our manuscript. Please find below a point-by-point response to the mentioned concerns. With kind regards and on behalf of all authors,

Rainer Kiko.

Response to Anonymous Referee # 1:

Referee Comment 1: Were measurements of respiration and ammonia excretion made separately or simultaneously? This is important for readers who are interested in cal-

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culating O:N ratios from the authors' data.

Response 1: Respiration and ammonia excretion measurements were made simultaneously. This will be clarified in the revised version and the data description accompanying the archived data. See also Response 2.

Referee Comment 2: Compared Figs 3 and 4 (combination of 2 temperature levels and 3 air-saturation levels) with those in Figs 5 and 6 (2 temperature levels and 4 air-saturation levels), the number of data in the former are significantly less than those in the latter. For example, the data number of *Undinula vulgaris* at 10% air-saturation and at 11°C is 5 (1+4, Fig. 2), but that of the same species at the same air-saturation and at the same temperature in Fig. 5 is far more greater than 5. Please clarify.

Response 2: As noted above, respiration and excretion rate measurements were conducted in parallel. Excretion rate measurements were realized as start – stop measurements in winkler type glass bottles. These incubation bottles were equipped with oxygen sensors that allowed the parallel measurement of the respiration rate. Respiration rates were determined as the decrease in oxygen in the incubation bottle after an initial acclimation phase (Reply fig. 1). As the oxygen level declined in the incubation bottle over time, we could determine respiration rates for different oxygen levels. For each experiment, temperature, initial oxygen level and initial CO₂ level were adjusted. To analyze if CO₂ has an effect on the excretion or respiration rates, the excretion rate (obtained for the entire time frame of the experiment) and the mean respiration rate for the entire time frame of the experiment were analyzed and plotted in Fig. 3 and 4. To analyze the effects of oxygen, the respiration rate data were analyzed at higher resolution to characterize the threshold at which the environmental oxygen level impacted the respiration rate. Therefore respiration rates for different oxygen levels within an experimental run were determined and the respiration rate data in Fig. 5 and 6 is presented at higher resolution. We will explain this procedure more clearly in a revised version of the manuscript.

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Referee Comment 3: Among 4 zooplankton tested, *Undinula* is non-migrant epipelagic copepod therefore is served as "control" which never experience temperature as low as 11°C and under-saturation of O₂. Borne this in mind, compared with *Pleuromamma* this species exhibit no distinct features in the performance in respiration and ammonia excretion across O₂ pressure tested at 23°C. What is the explanation for this?

Response 3: This might be due to the fact that the temperature within OMZs is usually rather low. Therefore, OMZ species might not be adapted to thrive at low oxygen levels and high temperatures, possibly explaining the similarity of an OMZ and a non-OMZ species in responding to low oxygen levels at high temperatures. However, with only one datapoint, it is difficult to discuss this hypothesis. In a revised version, we will point out that the determination of respiration and excretion rates over a wider temperature range could also be helpful to better understand and describe differences in performance between migratory and non-migratory species.

Referee Comment 4: Incorporation of pO₂ as a parameter of predictive models of respiration rate or ammonia excretion rate of diel migrating zooplankton into OMZ is not an easy task since the change in pO₂ is more or less correlated closely with other parameters such as temperature, depth distribution, and time of the day. Perhaps, previous workers might be well aware complex interactions (known as "multicollinearity") between these parameters.

Response 4: We agree with the reviewer and in a revised version will propose that other parameters (time of day, depth distribution etc.) also require further study to get to a more refined predictive model of respiration and excretion rates of diel migrating zooplankton into OMZs.

Referee Comment 5: The format of Species column of Table 3 is inconsistent. Please correct.

Response 5: The formatting will be corrected. The species name will be given in each row of the table.

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Response to Anonymous Referee # 2:

Referee Comment 1: The abstract needs to better reflect the work of the authors. At present much of the abstract is of an introductory nature with only the last 1 or 2 sentences reporting on the work/findings of the authors.

Response 1: We will rework the abstract in a revised version to include more results of our study.

Referee Comment 2: Of some concern is the low number of experimental animals in many of the treatments in determining respiration and ammonium excretion rates at different temperatures, air saturation and CO₂ -levels. In many cases the bars in figures 3 and 4 reflect the means of 3 animals [the authors need to comment on this].

Response 2: We will comment on the low number of n in some tests of the pCO₂ impact in the revised version of the manuscript. As noted in the discussion, we provide an estimate for the approximate difference in respiration rates that would have been necessary to result in their detection and also tested the impacts of pCO₂ in a generalized linear model. We would like to point out that 14 out of 16 (respiration) and 11 out of 16 (ammonia excretion) tests had high enough n to allow for t-testing and only in two cases significant effects were found. Furthermore, the results of the generalized linear model analysis are consistent with the results presented in figures 3 and 4. As discussed in the manuscript, the overall result of our analysis is that changes in pCO₂ have no significant effects on respiration or ammonia excretion rates of the species tested.

Referee Comment 3: The authors may consider revising the presentation of the data in figures 3 and 4 in the absence of any significant difference in the CO₂ plus and CO₂ minus treatments [and the low n]. At the very least better organization of the panels in figures 3 and 4 [by grouping panels of the same species] may facilitate easier reading/comparison. Better use of colour may also facilitate easier comparison.

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Response 3: We will revise the figures to show the significant differences in red colour and the non-significant tests in white. We believe that this will help to transfer the message that there are no consistent significant differences between the CO₂-plus and CO₂-minus treatment. The figure captions will be changed accordingly. We will furthermore organize the figures as 4 row * 5 column panels with each species in a row, with low temperatures left and high temperatures right and air saturation increasing from left to right. Information on the ordering logic will be provided in the figure caption.

Referee Comment 3: I also suggest repeating the labels "air saturation" and "oC" in each panel.

Response 3: The labels "air saturation" and "oC" will be repeated in each panel.

Referee Comment 4: The authors should also consider combining figures 5 and 6 owing to the almost identical and very lengthy figure captions.

Response 4: We would not like to combine these two figures, as this would require the single plots to be smaller and make it difficult to discern the symbols. Furthermore, the lower temperatures tested for copepods and krill do not coincide. We think that it is better to stress this fact by keeping the figures separate.

Referee Comment 4: Also the x-axes need to be labeled and ticks should be included on the x-axes of the upper panels in both figures 5 and 6.

Response 4: x-axes will be labeled and ticks included on the x-axes of the upper panels of figures 5 and 6.

Referee Comment 5: I also believe that the inclusion of a "Conclusion" will assist in focusing the reader on the findings of the study.

Response 5: We decided to present conclusions at the end of each of the three main paragraphs of the discussion (please see chapter 4.1: Page 17346, Line 21 to 23; chapter 4.2: Page 17347, Line 17 to 26; chapter 4.3: Page 17349, Line 15 to Page 17350 Line 15). We will make it more clear that these are the major conclusions of our

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manuscript by using formulations like "We conclude . . ." in the revised version of our manuscript, but would like to omit a repetition of these conclusions in a special chapter.

Minor comments:

Pg 17330 line 6: possibly delete "depth" Response: Will not be changed, as it is important to note that in this context the redistribution of the migrators along the depth axis is important.

line 13: replace "at" with "in" or "within" Response: We would not like to change this. We don't mean the regulation within the OMZ, but the regulation at OMZ conditions.

Pg 17331 line 25: replace "impact" with "influence" Response: "impact" will be replaced with "influence"

Pg17332 line 7: revise to "OMZs have expanded: : :." Response: Will be revised.

Pg17332 line 9: revise to "An expansion of OMZs : : : : ecosystems. For example: : :." Response: Will be revised.

Pg17332 line 13: revise to "Many organisms have adapted by developing enhanced: : :." Response: We would like to keep the formulation as it is, as an enhanced oxygen uptake capacity is not the only adaptation to enable OMZ survival in the OMZ.

Pg17332 line 25: revise to "Many species inhabiting the OMZ have evolved: : :." Response: Will be revised.

Pg17333 line 4: not sure that "constrained" is the right word here Response: We will change "constrained" to "described"

Pg17333 line 6: should "is first of all" be replaced with "primarily" Response: "is first of all" will be replaced with "primarily"

Pg17336 line 4: revise to "Animals were not fed before or during experiments." Response: Will be revised.

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line 25: revise to “0.2 μ m Whatman GFF filter” Response: Will be revised.

Pg17337 line 7: revise to “were treated with” Response: The sentence will be revised to “Antibiotics (25 mg L⁻¹ ampicillin and 25 mg L⁻¹ streptomycin) were added to the stocks after equilibration to inhibit microbial activity.”

line 10: what is meant by “immediately on board”? Response: This information is provided to clarify that pH and salinity were measured immediately after stock preparation, whereas the DIC and TA measurements were conducted in the home laboratory. The information that DIC and TA measurements were done in the home laboratory will be added to the manuscript.

Pg17338 line 14: revise to “and the recording of data for the determination of respiration rates: : :” Response: Will be revised.

Pg17338 line 21: revise to “obtained over a maximum incubation time of 16h” Response: Will be revised.

Pg17338 line 24: revise to “Samples were incubated for 2 to 10 h for the measurement of ammonium concentrations.” Response: Will be revised to “Animals were incubated for 2 to 10 h for the measurement of ammonium excretion rates.”

Pg17339 line 14: revise to “as the difference between the first and second measurement and the oxygen concentration was calculated as the mean of the initial and end oxygen concentrations” – not sure what the latter part of the sentence means.

Response: We will revise the sentence as suggested. The last part of the sentence explains that for each ammonium excretion rate measurement conducted at very low oxygen levels, we also needed to determine the oxygen concentration at which the measurement was taken, which is lower than the initial pre-set O₂ level, but higher than the final level. The respiration rates at very low oxygen levels are not linear and we therefore decided to take the mean of the initial and end values to provide a conservative estimate of the oxygen concentration during the excretion incubation.

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Pg17340 line 3: revise to “Pairwise t tests were employed to compare the respiration and excretion rates of the two pCO₂-treatments maintained under similar temperature and pO₂ conditions.” Response: Will be revised.

Pg17341 line 14: not sure what is meant by the sentence “Differences to the simulated environmental conditions were even larger: : :” Response: In line 13 we state that a treatment of seawater with a pure O₂/N₂ mix already reduces the pCO₂ and increases the pH of seawater in comparison to the levels observed in water equilibrated with normal air. Therefore, bubbling with oxygen or oxygen/nitrogen mixes leads to perturbations of the CO₂ system that could be important in experiments that only aim to test effects of hypoxia. In our case, the goal of the experiment was the realistic adjustment of CO₂ levels to those of the Atlantic and Pacific OMZ. Our results show that if such an adjustment is the goal, the usage of O₂/N₂ mixes is even more inappropriate. We will change this sentence to “Differences to the respective simulated OMZ conditions were even larger . . .”

Pg17341 line 18: revise to “were slightly above those of the ETSP OMZ.” Response: Will be revised.

Pg17344 line 15: revise to “at temperatures, and oxygen and carbon dioxide levels” Response: Will be revised.

Pg17345 line 11: revise to “experienced by the animals when migrating” Response: Will be revised.

Pg17345 line 25: should it not read “no significant changes” – otherwise “acute” needs to be quantified Response: Will be revised to “no significant changes”.

Pg17346 line 24: delete “these experience” Response: Will be changed to “the animals experience”.

Pg17348 line 3: revise to “Several studies have assessed: : :” Response: Will be revised.

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Pg17348 line 7: revise to “were obtained under mildly hypoxic to normoxic conditions”
Response: Will be revised.

Pg17348 line 9: revise to “For OMZs demonstrating only mild hypoxia, such as the ETNA OMZ, this approach: : :.” Response: Will be revised.

Pg17350 line 1: not sure what “[: :.]” means Response: As indicated by the quotation marks, the remark in between the quotation marks is a direct quote from Cocco et al. 2013. We shortened this quote for clarity, but will provide the entire quote in the revised manuscript. The entire sentence reads: “projections of the evolution of low O₂ regions will vary among models and be affected by large uncertainties.

Interactive comment on Biogeosciences Discuss., 12, 17329, 2015.

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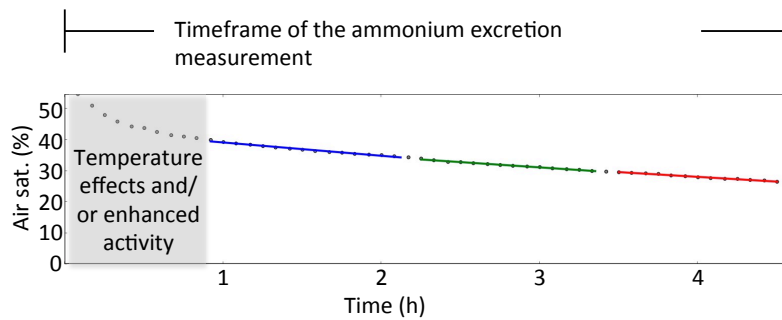


Fig. 1. Example for the analyses of respiration and excretion rate data. Slopes of blue, green and red linear regression lines represent respiration rate at a mean air saturation of 36, 31 and 27%, respective

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