

Dear Dr. Grégoire,

Thank you very much for handling the editorial process of our manuscript. Please find below our point-by-point answers to the reviewer's comments. We have reworked Figures 3 and 4, added further results and conclusions to the abstract and a dedicated conclusions paragraph to the article to improve the form of the article. I hope that we have addressed all points raised sufficiently.

With kind regards,

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 calculating O:N ratios from the authors' data.

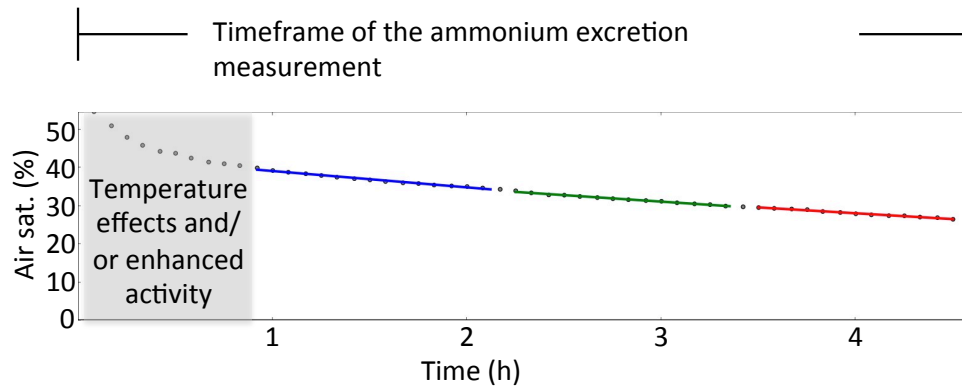
Response 1: Respiration and ammonia excretion measurements were made simultaneously. This was clarified in the revised version in line 230 and 278. See also Response 2.

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 CO<sub>2</sub> level were adjusted.

To analyze if CO<sub>2</sub> 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. This procedure is described in lines 337 to 342. 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 have added another sentence in lines 342 to 345 to explain this procedure.



Reply Fig. 1

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 11oC and under-saturation of O<sub>2</sub>. Borne this in mind, compared with *Pleuromamma* this species exhibit no distinct features in the performance in respiration and ammonia excretion across O<sub>2</sub> pressure tested at 23oC. 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 the revised version, we point out that the determination of respiration and excretion rates over a wider temperature range could also be helpful to better understand differences in performance between migratory and non-migratory species. Lines 556 to 559.

Referee Comment 4: Incorporation of pO<sub>2</sub> 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 pO<sub>2</sub> 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 we now propose that also other parameters (time of day, depth distribution etc.) require further study to get to a more refined predictive model of respiration and excretion rates of diel migrating zooplankton into OMZs (Lines 559 to 561). However, we would like to point out that on a global scale the parameters pO<sub>2</sub>, temperature and depth distribution do not covary. Oxygen levels found at 300 m depth in the ETNA are very different from those found at the same depth in the ETSP and also the migration depth varies (about 230 m depth in the ETSP; about 400 m depth in the ETNA; own ADCP Data observations of the migration)

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

Response 5: The formatting was corrected. The species name is now given in each row of the table.

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: The abstract was reworked 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 CO<sub>2</sub> -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 now comment on the low number of n in some tests of the pCO<sub>2</sub> impact in lines 478 to 481 of the revised version of the manuscript. We also 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 pCO<sub>2</sub> 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 pCO<sub>2</sub> 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 CO<sub>2</sub> plus and CO<sub>2</sub> 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.

Response 3: We revised the figures to now show the significant differences in red colour and the non-significant tests in white. We believe that this helps to transfer the message that there are no consistent significant differences between the CO<sub>2</sub>-plus and CO<sub>2</sub>-minus treatment. The figure captions were changed accordingly. We furthermore organized 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 was provided in the figure caption.

Referee Comment 3: I also suggest repeating the labels “air saturation” and “°C” in each panel.

Response 3: The labels “air saturation” and “°C” are now 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 choose to not 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 were 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 present conclusions at the end of each of the three main paragraphs of the discussion, but now also included a more general conclusion chapter at the end of the manuscript.

Minor comments:

Pg 17330 line 6: possibly delete “depth” Response: We choose not to change this, as it is important to note that in this context the redistribution of the migrators along the depth axis is relevant.

line 13: replace “at” with “in” or “within” Response: We choose not 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” was replaced with “influence”

Pg17332 line 7: revise to “OMZs have expanded: : :.” Response: Was 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 kept 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: Revised.

Pg17333 line 4: not sure that “constrained” is the right word here Response: We changed “constrained” to “described”

Pg17333 line 6: should “is first of all” be replaced with “primarily” Response: “is first of all” was replaced with “primarily”

Pg17336 line 4: revise to “Animals were not fed before or during experiments.” Response: Revised.

line 25: revise to “0.2  $\mu\text{m}$  Whatman GFF filter” Response: Revised.

Pg17337 line 7: revise to “were treated with” Response: The sentence was 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 was added to the manuscript in line 291.

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

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

Pg17338 line 24: revise to “Samples were incubated for 2 to 10 h for the measurement of ammonium concentrations.” Response: 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 revised 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 O<sub>2</sub> 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.

Pg17340 line 3: revise to “Pairwise t tests were employed to compare the respiration and excretion rates of the two pCO<sub>2</sub>-treatments maintained under similar temperature and pO<sub>2</sub> conditions.” Response: Revised.

Pg17341 line 14: not sure what is meant by the sentence “Differences to the simulated environmental conditions were even larger: : :” Response: In the previous line we state that a treatment of seawater with a pure O<sub>2</sub>/N<sub>2</sub> mix already reduces the pCO<sub>2</sub> 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 CO<sub>2</sub> 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 CO<sub>2</sub> levels to those of the Atlantic and Pacific OMZ. Our results show that if such an adjustment is the goal, the usage of O<sub>2</sub>/N<sub>2</sub> mixes is even more inappropriate. We changed 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: Revised.

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

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

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

Pg17346 line 24: delete “these experience” Response: Revised to “the animals experience”.

Pg17348 line 3: revise to “Several studies have assessed: : :” Response: Revised.

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

Pg17348 line 9: revise to “For OMZs demonstrating only mild hypoxia, such as the ETNA OMZ, this approach: : :” Response: 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 in the previous version, but now provide the entire quote in the revised manuscript. The entire sentence reads: “projections of the evolution of low O<sub>2</sub> regions will vary among models and be affected by large uncertainties.