

Interactive comment on “Changing mineralogical properties of shells may help minimize the impact of hypoxia-induced metabolic depression on calcification” by Jonathan Y. S. Leung and Napo K. M. Cheung

Jonathan Y. S. Leung and Napo K. M. Cheung

jonathan_0919@hotmail.com

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In this paper, J. Leung and N. Cheung describe and discuss the effect of hypoxia on growth, shell properties and chemistry, respiration and feeding activity of *Hydroides di-ramphus*. Overall the paper is well written, the data are well presented and discussed but one aspect of the experimental design concerns me. The authors achieved incubation in hypoxia by “aerating seawater with a mixture of nitrogen and air” (l. 76-77). This technique indeed allows replacement of dissolved oxygen by N₂ but also tends to remove dissolved CO₂ and can then increase water pH. Did the authors measure and

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control pH in the different DO treatments? If yes, please provide this information. If no, I'm afraid that most of the discussion on the effect of hypoxia on shell chemistry might be irrelevant giving that changes in pH could explain the changes in calcite/aragonite and Mg/Ca. The lack of discussion of the effect of pH is especially surprising since you mention at least 6 times the ocean acidification in the introduction and none in the discussion. I would therefore recommend to further discuss the potential changes in pH during the experiment (ideally you should measure it) and how it could affect the calcification of this polychaete species.

RESPONSE: We can provide the pH data. The reviewer is right to state that pH was slightly elevated by pumping the mixture of nitrogen gas and air into the seawater. How basification of seawater affects Mg/Ca and calcite/aragonite remains largely unexplored, but can be inferred from studies on ocean acidification. In the context of ocean acidification, Mg/Ca in calcite would be reduced as high-Mg calcite is more susceptible to dissolution. Yet, the huge difference in Mg/Ca (hypoxia vs. normoxia) is unlikely only caused by the small difference in pH. Since Mg/Ca is mainly under biological control (Bentov and Erez, 2006), we suggest that hypoxia is the major factor causing the difference in Mg/Ca via its impact on energy metabolism. Calcite/aragonite could be altered in bimineralic calcifiers under ocean acidification (usually higher proportion of calcite) because aragonite will become limiting first due to higher solubility than calcite (Feely et al., 2004). However, this prediction cannot be applied to basification because both calcite and aragonite are saturated. We will discuss the potential pH effect in the revision as requested.

To summarize, I would recommend the publication of the present paper in the following conditions: -the authors detail if/how they controlled the pH during the experiment; - estimate or measure the pH changes during N₂ bubbling; - discuss of how could pH changes (if existing) could explain together with hypoxia the shell chemistry observations you made.

RESPONSE: We will address these points in the revision.

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In addition, here are some extra comments on the manuscript: Introduction section. In the introduction, you mention at least 6 times the ocean acidification, a phenomenon that you do not mention again at any point later in the paper. As explained above I think that you should discuss more of the effect of pH on calcification in this paper.

RESPONSE: We mentioned ocean acidification to highlight that calcification is very likely not related to carbonate chemistry in seawater according to recent studies; therefore, we suggested that calcification is mainly associated with physiological performance, which would be greatly impaired by hypoxia. We agree that ocean acidification was a bit overemphasised in the Introduction and will be tuned down in the revision.

Line 26. Add a reference for the hypoxia threshold. Also add the equivalent threshold in $\mu\text{mol/L}$ ($63 \mu\text{mol/L}$) to facilitate the understanding of readers more used to this unit.

RESPONSE: Suggestions will be adopted in the revision.

L. 33. Remove the successive closing and opening brackets (several occurrences in the whole paper).

RESPONSE: Suggestion will be adopted in the revision.

L. 42. The paper by Nardelli and coauthors is an experiment in anoxia not hypoxia, specify use as a reference.

RESPONSE: The sentence will be revised as "... calcification under hypoxia (Mukherjee et al., 2013; Keppel et al., 2016), and even anoxia (Nardelli et al., 2014)".

L. 53. Provide the species name.

RESPONSE: Species name will be added in the revision.

L. 67. Identify that this species lives fixed on hard substrate.

RESPONSE: This information will be added in the revision.

L. 77. "mixture of nitrogen and air" correct to dinitrogen and specify if possible the %

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ratio of the mixture.

RESPONSE: Suggestions will be adopted, except that we prefer to use "nitrogen gas" rather than "dinitrogen" as more readers can understand the former.

L.79. Specify the stability of the oxygen concentration.

RESPONSE: We will add a table to show the stability of O₂ concentration.

Section 2.1. Specify somewhere how long did the experiment last, and if there were some substrate in the aquariums.

RESPONSE: The experiment lasted for 3 weeks and there was no substrate in the aquarium.

L.87. "2 ml" change to "2 mL" and make sure that this unit is written in capital letters in the whole paper.

RESPONSE: We will check and correct this unit throughout the manuscript.

Section 2.2 specify if initial size were homogenized or individuals were randomly dispatched in the different DO levels/bottles.

RESPONSE: Before the exposure, we standardized the size of individuals which were then randomly assigned to the bottles. This information will be added in the revision.

L.105. 5 individuals randomly selected?

RESPONSE: Yes. Information will be added in the revision.

L.108. How and where do you measure the initial and final concentrations? Inside or outside of the syringe? In which volume? Do you shake the syringe prior to measurement (or water expelling) to homogenize the water? Is there some stirring inside the syringe? How do you seal the syringe? Could you add some references for this measuring method? If you do not have any water homogenization system, and oxygen concentration is only measured outside of the syringe, there might be some unmeasured

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“stratification” occurring. There is an important difference in oxygen concentration in between the water close to the syringe tip and the water close to the individuals.

RESPONSE: The initial and final DO concentrations were measured by inserting the DO probe into the tip of syringe which was sealed by Blu-Tack after initial measurement. The volume of seawater in the syringe was ~35 mL (Ln 129). For each measurement, we have to ensure that the DO concentration is uniform by gently stirring the seawater with the DO probe, which provides the real-time measurement of DO concentration. The reading was taken when the DO concentration is stable along the depth to ensure no “stratification”. It is not advised to constantly stir the seawater inside the syringe, which causes stress to the individuals. Relevant references are Zhao et al. (2011) and Leung et al. (2013). We will add more information in the revision for clarity.

L.110 Give some extra information about the blanks. What is the variation observed in these measurements? Does the hypoxic blank show an increasing oxygen concentration suggesting a potential leak due to the sealing system? In the hypoxic sets of measurements, what was the final oxygen concentration? Did the individuals survive to this oxygen concentration? Respiration rate being size dependent, precisely identify the size distribution of the different samples.

RESPONSE: The blank is used to correct the background change in DO concentration, which was negligible after the 1-hour exposure, including the hypoxic blank ($\pm \sim 1\%$). This indicates that the syringe was sealed properly. The final measurement for the hypoxic group was about 0.5 mg L⁻¹ and *H. diramphus* was able to survive after the measurement due to strong tolerance to hypoxia. Before the exposure, we standardized the body size to about 20 mm and randomly assigned the individuals to each bottle to avoid bias. Information will be added in the revision.

L.122 “+add-on” specify.

RESPONSE: “PERMANOVA+ add-on” is the name of software.

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L.142. “we demonstrated” at this stage of the stage of the discussion it is too early to assume that you have demonstrated anything.

RESPONSE: We will change “demonstrated” to “found”.

L. 144 and 160. I would recommend avoid using the term “adaptive” due to its potential evolutionary connotation.

RESPONSE: We will change “adaptive” to “beneficial, favourable, positive, etc.”.

L. 154. You mention quite often the “metabolic energy for calcification” is there any way to estimate it and compare it to the loss of energy provided by oxygen respiration? Palmer (1992) gives estimates in terms of J per mass of CaCO₃ produced, oxygen respiration rates can also be easily converted to joules (roughly 3000kJ per mole of glucose reduced). With such calculations, you could estimate the part of the respiration-energy required for calcification in both oxygenated and hypoxic conditions.

RESPONSE: We appreciate this comment. In this study, however, it is premature to estimate the actual metabolic cost of calcification as we did not measure the weight of CaCO₃ produced and ingredients of the shell (e.g. proteins and carbohydrates), which are species-specific. The energetics of calcification is beyond the scope of this study. Studying energetics is challenging (to us) since there are many parameters should be considered. Palmer’s model is good, but more parameters can still be added to provide an accurate estimation (see dynamic energy budget model).

L. 177. “the anti-predator response is not markedly affected by hypoxia”. Do you know who are the predators for this polychaete species and how these predators are sensitive to hypoxia? If the predators are sensitive and/or non-active in hypoxia, there might not be any point of being protected under such conditions.

RESPONSE: Predators are predatory fish, such as spinefoots. Yet, humans are responsible for the shell damage during the removal of *H. diramphus* from many man-made structures, which can happen irrespective of DO concentration. Anti-predator

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response is another topic in ecology and therefore we will change “anti-predator response” to “defensive response” or similar wordings to avoid confusing readers.

L. 197. “affecting shell solubility”. Precisely how does it affect the solubility (positively or negatively?).

RESPONSE: We will change “affecting” to “reducing” or other similar words.

L. 199. “it is predicted that metabolic energy is involved in the control of magnesium incorporation”. Is that your prediction or is it some other authors? Please identify the source/reasoning.

RESPONSE: It is our prediction but a previous study suggests that energy is involved in the control of magnesium incorporation (Bentov and Erez, 2006). We will elaborate in the revision.

L. 211. The citation to your other paper seems irrelevant here since in that paper you do not analyze the effect of hypoxia.

RESPONSE: Although the stressor is different, the concept in this paper is highly relevant and thus we wrote “see also” just to recommend reading another similar study.

Figure 1 caption. Provide the number of replicates on which your mean and S.E. are based on. Add the initial and final sizes (in Table 1 or supplementary data).

RESPONSE: Information will be added in the revision.

Table 1 caption. Provide the number of replicates on which your mean and S.E. are based on.

RESPONSE: Information will be added in the revision.

Table S1. This table can appear in the paper and not in the supplementary data.

RESPONSE: Suggestion will be adopted in the revision.

Explain what “MS” stands for. Give the degrees of freedom values.

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RESPONSE: We will change MS to “Mean square” and add df values in the revision.

References list. L. 254. I would not recommend keeping this citation since this paper was not accepted for publication.

RESPONSE: Even though this paper is not accepted, it has still been highly cited. We will decide whether this citation should be kept.

BGD Review criteria:

-Does the paper address relevant scientific questions within the scope of BG? Yes

-Does the paper present novel concepts, ideas, tools, or data? Yes

-Are substantial conclusions reached? Yes, but further discussions are needed

RESPONSE: We will further discuss the findings as suggested.

-Are the scientific methods and assumptions valid and clearly outlined? More details about the oxygen respiration method should be provided. The potential effect of pH should be further discussed.

RESPONSE: We will describe the O₂ measurement clearly as requested and discuss the potential pH effect.

-Are the results sufficient to support the interpretations and conclusions? No, more information about pH should be provided.

RESPONSE: We will provide pH data in the revision.

-Is the description of experiments and calculations sufficiently complete and precise to allow their reproduction by fellow scientists (traceability of results)? Partially, more information about the respiration measurements should be provided.

RESPONSE: More information will be provided in the revision.

Do the authors give proper credit to related work and clearly indicate their own

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new/original contribution? Yes

-Does the title clearly reflect the contents of the paper? Yes

-Does the abstract provide a concise and complete summary? Yes

-Is the overall presentation well structured and clear? Yes

-Is the language fluent and precise? Yes

-Are mathematical formulae, symbols, abbreviations, and units correctly defined and used? Yes, authors just need to homogenize the writing of "liters" unit.

RESPONSE: We will make this unit consistent in the revision.

-Should any parts of the paper (text, formulae, figures, tables) be clarified, reduced, combined, or eliminated? Supplementary table should appear in the paper. The potential effect of other variables than hypoxia (e.g. pH) should be further discussed.

RESPONSE: Suggestions will be adopted in the revision.

-Are the number and quality of references appropriate? Yes

-Is the amount and quality of supplementary material appropriate? Yes

References

Bentov S and Erez J (2006) Impact of biomineralization processes on the Mg content of foraminiferal shells: A biological perspective. *Geochem. Geophys. Geosyst.* 7, Q01P08.

Feely RA et al. (2004) Impact of anthropogenic CO₂ on the CaCO₃ system in the oceans. *Science* 305, 362–366.

Leung YS et al. (2013) Physiological and behavioural responses of different life stages of a serpulid polychaete to hypoxia. *Mar. Ecol. Prog. Ser.* 477, 135–145.

Zhao Q et al. (2011) Effects of starvation on the physiology and foraging behaviour of

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two subtidal nassariid scavengers. *J. Exp. Mar. Biol. Ecol.* 409, 53–61.

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