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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

Anonymous Referee #1

Received and published: 1 May 2017

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 diramphus.

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 control

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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.

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.

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.

Line 26. Add a reference for the hypoxia threshold. Also add the equivalent threshold in μmol/L (63 μmol/L) to facilitate the understanding of readers more used to this unit.

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

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

L. 53. Provide the species name.

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

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

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

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

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

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

L.105. 5 individuals randomly selected?

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 “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.

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.

L.122 “+add-on” specify.

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

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

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.

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.

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

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.

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

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).

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

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based on.

Table S1. This table can appear in the paper and not in the supplementary data. Explain what “MS” stands for. Give the degrees of freedom values.

References list.

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

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

-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.

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

-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.

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

-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.

-Are the number and quality of references appropriate? Yes

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

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