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Biogeosciences Discussions

Interactive comment on "Calcification and inducible defence response of a calcifying organism could be maintained under hypoxia through phenotypic plasticity" *by* Jonathan Y. S. Leung and Napo K. M. Cheung

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The manuscript by Leung and Cheung provides information regarding how calcification processes in polycheate worms could be influenced by future hypoxia. The results are pretty straight forward, and I consider these types of studies are important, although not ground-breaking. I have a few concerns that should be addressed before this manuscript could be accepted.

RESPONSE: We are pleased to see that the reviewer recognizes the importance of

this work.

The grammar and style should be improved in the introduction and discussion of this m/s before it could be published in any outlet. There are too many examples of this for me to highlight every one, but for example the use of the term "defence response". I suggest the authors ask a senior colleague who is a native English speaker to read over and correct for them. In general there is also a lot of speculation for a 21 day long study.

RESPONSE: Being senior English writers, we believe that the overall quality of English writing is good enough for publication based on our experience. Yet, we will polish the writing in the revision to maximize readability. The term "defence response" is widely used in the literature to describe the defensive behaviours of organisms at both individual (e.g. anti-predator response) and tissue/cellular (e.g. immune response) levels.

Specific comments: Line 40: In general I agree that calcification costs energy, but in some organisms the energy-dependence has been postulated as low (e.g. in corals – see McCulloch et al. (2012)). So this may be true for gastropods, but not necessarily so for some other organisms. So this sentence needs to be balanced somewhat.

RESPONSE: Please note that McCulloch et al. (2012) examined the effect of ocean acidification, where the "low energy cost" only refers to the additional energy for pH regulation at the calcification site, while the energy cost of calcification (i.e. production of calcified structures) is not taken into consideration. Thus, this "low energy cost" is irrelevant to our study because hypoxia does not affect the acid-base balance of organisms.

Line 70: The hypotheses around phenotypic plasticity needs to be strengthened and clarified. What exactly is the phenotype that is plastic here? The capacity to form different types of mineral in the shell? Or simply that responses will differ between control and reduced O2 concentrations? Reading the discussion, I think the authors

are misusing the term phenotypic plasticity. Demonstrating variability in responses of individuals within the same population to a stressor is not demonstrating phenotypic plasticity, nor is demonstrating a different response under different treatments between different individuals.

RESPONSE: In ecology, “phenotypic plasticity” means that individuals can change their phenotypic traits (e.g., growth, behaviour, shell properties, etc.) in response to altered environmental and biological conditions, which has adaptive values (Malausa et al., 2005). In this study, the mineralogical properties of shells were proven to be plastic phenotypic traits as they were modified in response to hypoxia. The term “phenotypic plasticity” may be a bit general because the phenotypic traits in this study were only associated with mineralogical properties. To be more explicit, we will replace “phenotypic plasticity” with “mineralogical plasticity” in the revision (Leung et al., 2017).

Leung, J.Y.S., Russell, B.D., and Connell, S.D.: Mineralogical plasticity acts as a compensatory mechanism to the impacts of ocean acidification. Environ. Sci. Technol., 51, 2652–2659, 2017.

Malausa, T., Guillemaud, T., and Lapchin, L.: Combining genetic variation and phenotypic plasticity in tradeoff modelling. Oikos, 110, 330–338, 2005.

Line 82: How was pH measured, and on what scale, using what buffers? More information needed here. How was salinity and temperature measured? I see some of these details in table S1, but there are required in the methods section.

RESPONSE: The pH was measured on NBS scale by a pH meter, calibrated using NBS buffers (Table S1). Temperature and salinity were measured using a thermometer and refractometer, respectively (Table S1). Since they are not the key parameters in this study, it is better to keep them in the Supplementary Information.

Statistical analysis: why use a permanova? I would expect each parameter to be separated analysed using univariate analyses as a first step. A justification for using

permanova over an anova or linear model needs to be justified here.

RESPONSE: Please note that PERMANOVA can be used for univariate analysis, as applied in our study. With the use of Euclidean distance matrix, PERMANOVA can produce the same F-statistics as traditional ANOVA (Anderson, 2001).

Anderson, M.J.: A new method for non-parametric multivariate analysis of variance. Aust. Ecol., 26, 32–46, 2001.

Line 191-192: But this statement is at odds with the findings of the permanova and the figures, that calcification was impacted by hypoxia in this study. Also, the end of the sentence that this could be due to phenotypic plasticity needs to be explained, as this makes no sense to me.

RESPONSE: At this point of discussion (1st paragraph), we only make a general summary of the findings, which can help readers quickly grasp the key message of this study. The detailed explanation for each finding is provided in the subsequent paragraphs. We can revise the statement about the impact of hypoxia on calcification for clarity.

References used in the review: McCulloch MT, Falter J, Trotter J, Montagna P (2012) Coral resilience to ocean acidification and global warming through pH up-regulation. Nature Climate Change, 2, 623-627.

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