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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

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There are still limited studies about the influence of oxygen-depleted environment on carbonate shell formation. The reviewer can believe this study of Leung and Cheung give important knowledge on this on-going problem. The contents of consideration are consistent with the interests of the Biogeosciences' audience.

RESPONSE: Thank you very much for your interest in our manuscript.

On the other hand, there are some remained questions about the results and consid-

erations. The authors should show the actual sampling/measurement points on the specimens with images. Chan et al. (Journal of Structural Biology 189 (2015) 230-237) reports the shell formation of H. elegans (belonging to the same genus of H. diramphus). According to the study, calcite/aragonite ratio and Mg/Ca altered by ontogeny. Mineralogical heterogeneity was also found in the single shell wall by Chen et al. (2015). The reviewer expects, then, similar variability may be found in H. diramphus, too. The authors seems working with the species for long time. By their knowledge, the ontogenic mineralogical and Mg/Ca variation of H. diramphus should be shown by this study or previous studies. Possibly, the current interpretations are totally revised.

RESPONSE: We appreciate this comment. As highlighted in the Introduction and Title, however, this study aims to examine whether calcifying organisms can modify mineralogical properties (i.e. mineralogical plasticity) to possibly accommodate hypoxia, which has great research interest and novelty in physiological ecology. To answer this ecological question, mineralogical properties and physiological performance are needed. Technically, we used the whole newly-produced shells for the analysis of mineralogical properties (Ln 96-97), which can show the overall change in mineralogy that we are interested in. The developmental process of shells (or ontogeny) is beyond the scope of this study, but we can provide SEM images of shell ultrastructure to show whether there are significant changes in the morphology or density of carbonate crystals among treatments, which may be associated with mechanical strength.

Though authors have considered that calcite/aragonite ratio would depend on energy requirements/consumptions, the ratio is also influenced by magnesium composition and organic compound compositions of mother fluid of calcification, as well as temperature, pressure etc (e.g. Berner, Geochim et Cosmochim Acta 1975, 489-504; Meldrum and Cölfen, Chem. Rev., 2008, 4332-4432). The reviewer expect that the energy for shell formation would be spent on production of the organic compounds of calcification substrate and enzymes, too (ion pumps, carbonic anhydrase etc.). The authors should show the amount of organic matter in the shell.

RESPONSE: We also recognize that some environmental factors can affect mineralogical properties. In this study, however, we only manipulated two factors (dissolved oxygen and shell damage), while other factors remained unchanged, meaning that the change in mineralogical properties is caused by these two factors (i.e. sources of variation). Organic matter content in the shell is highly related to mechanical properties; therefore, we analysed it few days ago as requested, and the result will be added in the revision.

It will be also nice to show the enzyme activity during shell formation for further consideration if possible.

RESPONSE: Enzymatic activity (e.g. carbonic anhydrase) or other molecular responses during shell formation are beyond the scope of this study, but can be examined with ontogeny in future investigation to elucidate the underlying mechanisms causing different shell properties or growth.

Though the authors thought that the hypoxia condition has increased relative amorphous calcium carbonate (ACC) content by its lower energy demand, according to their cited Weiner and Addadi (1997), it is described that it requires much energy to maintain ACC. There are a discrepancy with authors' consideration.

RESPONSE: The reviewer might have misunderstood our idea. We first need to recognize that stabilization of ACC requires metabolic energy, regardless of DO concentration (i.e. same energy cost between normoxia and hypoxia). Yet, crystallization of ACC to form calcite or aragonite requires extra metabolic energy. The higher relative ACC content indicates that the animals were reluctant/unable to allocate extra metabolic energy to crystallization, possibly due to the reduced energy budget under hypoxia.

On the other hand, Fig 1 clearly show authors basic understanding about depression of calcareous shell by low-DO condition. In the hypoxic condition, shell growth slows and hardness increases. This key findings is valuable to publish. It can be understood that this may increase the volume by decreasing the density or the like in order to promote

growth in the normal oxygen condition in my mind.

RESPONSE: We appreciate reviewer's attempt to interpret the finding. The reduced shell growth under hypoxia is not surprising as it has been previously demonstrated. The most exciting finding is the maintenance of rapid shell growth plus production of harder shells under hypoxia, when the shell was damaged. This unexpected finding suggests that H. diramphus is tolerant to hypoxia and able to allocate more energy to the defensive response, despite the weakened aerobic metabolism. This response is vital for survival.

From the above, the result of the authors can be regarded as insufficient to support their own hypothesis. Some additional measurements and restructure of whole manuscript will be necessary to evident authors' current consideration. I would like to recommend resubmission with additional data and discussion.

RESPONSE: This reviewer is probably a specialist in structural biology and wants to see more technical details. Physiological ecology, however, concerns how environmental stressors impact the fitness and survival of organisms and whether/how organisms can adapt to altered conditions. As such, studies on climate change biology are mainly based on the overall response of organisms to stressors (please see some of the ecological studies below). After careful consideration, we will provide SEM images and organic matter content data in the revision, which are relevant and can greatly strengthen this manuscript.

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