Dear Editor,

The manuscript “Calcification and inducible defense response of a calcifying organism could be maintained under hypoxia through phenotypic plasticity” by Leung and Cheung raises a few interesting questions regarding organismal adaptations to hypoxia. Although authors present a few interesting ideas, I do not find the manuscript in its current form suitable for publication due to a significant shortfall in methods description, exaggerated interpretation of results and an incoherent discussion. However, if the Discussion paper manuscript progresses for publication in Biogeosciences, I suggest that the authors consider some major revisions that I have described in detail in the supplement. Please find attached my suggestions for major and minor revisions.

RESPONSE: We thank this reviewer for reviewing and giving suggestions for our manuscript. We found that most of the comments are related to the methods and can be clarified easily. The only two comments for the discussion section are minor and can be easily addressed as well. Therefore, we are happy to incorporate the good suggestions into the revision.

Major Comments:

Title: I do not find this title representative of the authors results/discussion. Please describe which aspects of the phenotype are considered plastic, since there is no change in mechanical strength and authors discuss less crystallographic control and magnesium regulation under hypoxic conditions.

RESPONSE: The plastic traits are the mineralogical properties of shells. We will make the title more descriptive in the revision.

Lines 34-38: Authors seem to go around a point here. Please state exactly what drives calcification instead of pH and seawater chemistry.

RESPONSE: The proposed main driver for calcification is the energetics of organisms (Ln 38-39, 41-42), which can be substantially affected by hypoxia (Ln 39-43).

Line 54: Energy costs can increase rather than reduce, if organic content is modified such that it increases.

RESPONSE: The word “modify” does not indicate the direction of change. We will change it to “decrease” to avoid ambiguity.

Line 76: Mean size of polychaetes?

RESPONSE: The tube length of polychaetes mostly ranged from 35 to 45 mm. This will be added to the revision.

Line 102: Diameter of hole drilled?

RESPONSE: About 2 mm. This will be added to the revision.

Line 103: Tubes were glued using what?

RESPONSE: Hot-melt adhesive. This will be added to the revision.

Line 114: How was the shell fragment acquired and cleaned of organic tissue?

RESPONSE: Shell fragments were obtained by breaking the newly-produced shells using a pair of forceps and they were then cleaned by rinsing with deionized water. Please note that the flesh of polychaetes does not attach to the shell. This information will be added to the revision.

Line 116: Was the same surface (e.g: inner shell surface) always used for indentation?

RESPONSE: Yes, we always used the inner shell surface for indentation. This will be clarified in the revision.

Line 122: Organic content of shell or whole polychaete? It is of the shell I assume, but is unclear.

RESPONSE: Only the newly-produced shells were used for analysis of organic matter content. We will make this sentence clearer.

Line 126: How was the shell powder acquired?

RESPONSE: Shell powder was obtained by grinding the newly-produced shells using a mortar and pestle. This will be clarified in the revision.

Line 130: Please provide information on how these polymorphs are typically distributed in the organism. Are the polymorphs specific to the outer/inner layer of the shell?

RESPONSE: We do not have information on the distribution of these carbonate polymorphs. Yet, it is not necessary to know that because it is irrelevant to our research question.

Line 139: Were ACC, aragonite and calcite standards measured? Please explain why, if not.

RESPONSE: Standards are used for determination of absolute quantity, but only relative quantity of these parameters is needed in our study. Specifically, since relative ACC content is indicated by the peak ratio in the IR spectrum, it is not necessary to measure the standard as long as background calibration for the baseline is made (Chan et al., 2012; Leung et al., 2017). As for the calcite to aragonite ratio, we apply the calibration equation in a method paper (Kontoyannis and Vagenas, 2000), which is derived by using pure calcite and aragonite.

Chan, V.B.S., Li, C., Lane, A.C., Wang, Y., Lu, X., Shih, K., Zhang, T. and Thiyagarajan, V: CO_2-driven ocean acidification alters and weakens integrity of the calcareous tubes produced by the serpulid tubeworm, *Hydroides elegans*. PLoS ONE, 7, e42718, 2012.

Leung, J.Y.S., Connell, S.D., Nagelkerken, I. and Russell, B.D.: Impacts of near-future ocean acidification and warming on the shell mechanical and geochemical properties of gastropods from intertidal to subtidal zones. Environ. Sci. Technol., 51, 12097–12103, 2017.

Kontoyannis, C.G. and Vagenas, N.V.: Calcium carbonate phase analysis using XRD and FT-Raman spectroscopy. Analyst, 125, 251–255, 2000.

Line 140: Diameter of the KBR-shell powder disc?

RESPONSE: About 2 cm. This information will be added to the revision.

FTIR: FTIR is a bulk measurement and ideally should not be used to infer "relative" proportions of carbonate polymorphs. Typically, the presence of a 713 cm-1 peak is indicative of crystalline calcium carbonate comprising the bulk of shell carbonates. However, I am aware that this interpretation has been used before and if authors proceed with the analyses, could they please clarify if the spectra were scaled so that 713 cm-1 peaks had the same heights as described in Weiss et al (2002)? In addition, please specify the typical size of crystallites in shell since such ratios have been demonstrated to be influenced by particle size (Kristova et al 2015).

RESPONSE: FTIR has been widely used to indicate the relative ACC content by measuring the peak ratio between 856 cm^{-1} and 713 cm^{-1} (e.g. Beniash et al., 1997; Chan et al., 2012; Leung et al., 2017). Since relative ACC content is indicated by this ratio rather than an absolute peak height, it is not necessary to rescale the peak height at 713 cm^{-1} to that in Weiss et al. (2002). The particle size of shell powder was ~5 µm.

Beniash, E., Aizenberg, J., Addadi, L., and Weiner, S.: Amorphous calcium carbonate transforms into calcite during sea urchin larval spicule growth. Proc. R. Soc. B, 264, 461–465, 1997.

Line 148: What were the syringes made to of?

RESPONSE: Polypropylene plastic. We will add this information in the revision.

Line 156: Hunger is only standardised if individuals were at the same start point.

RESPONSE: Therefore, all individuals were starved for 1 day prior to feeding trials. This is more than enough for them to clear their gut content.

Lines156-166: This doesn't represent clearance rates during the experiment.

RESPONSE: We disagree. This clearance method has been widely applied for determination of clearance/filtering/feeding rate of feeding feeders (e.g. Riisgård, 2001; Contreras et al., 2012; Leung et al., 2013; Leung and Cheung, 2017).

Contreras, A.M., Marsden, I.D., and Munro, M.H.G.: Effects of short-term exposure to paralytic shellfish toxins on clearance rates and toxin uptake in five species of New Zealand bivalve. Mar. Freshw. Res., 63, 166–174, 2012.

Leung, J.Y.S. and Cheung, N.K.M.: Feeding behaviour of a serpulid polychaete: Turning a nuisance species into a natural resource to counter algal blooms? Mar. Pollut. Bull., 115, 379–382, 2017.

Leung, Y.S., Shin, P.K.S., Qiu, J.W., Ang, P.O., Chiu, J.M.Y., Thiyagarajan, V., and Cheung, S.G.: Physiological and behavioural responses of different life stages of a serpulid polychaete to hypoxia. Mar. Ecol. Prog. Ser., 477, 135–145, 2013.

Riisgård, H.U.: On measurement of filtration rates in bivalves — the stony road to reliable data: review and interpretation. Mar. Ecol. Prog. Ser., 211, 275–291, 2001.

Line 170-180: Please provide full FTIR spectra as a supplementary figure.

RESPONSE: We can provide a FTIR spectrum as a supplementary figure.

Lines 267-268: Can inferences be made regarding whether inner/outer layers were calcified if the polymorphs are specific to a layer of the polychaete shell?

RESPONSE: We cannot make this inference based on our results. As mentioned above, further investigation on structural properties is needed to answer this question, which is beyond the scope of this study.

Line 233-234: This is a strong statement. Regulation of Mg may be interpreted but the authors results do not signify that it is relaxed under hypoxia.

RESPONSE: We will tone down this statement, as suggested by another reviewer.

Line 256-257: Please delete this final sentence. It is a very strong statement and the whole paragraph does not explain why hypoxia the key stressor in the future (which is debatable anyway).

RESPONSE: We will delete this sentence.

Minor Comments:

Line 10-11: Sentence like this needs a reference.

RESPONSE: In the Abstract, citations should be avoided.

Line 25: change "shells" to skeletons.

RESPONSE: We will replace “shells” with “shells or skeletons”.

Line 32: Delete "however".

RESPONSE: Suggestion will be adopted.

Line 368: Please provide full reference.

RESPONSE: We will follow the journal’s latest style.

Figure 5 (SEM): Are these images of the aragonite or calcitic parts of the shell? The legend needs more descriptive text. It is not obvious to me how these images indicate shell integrity.

RESPONSE: We cannot identify the type of carbonate mineral based on these images and this is beyond the scope of this imaging analysis. We will elaborate the figure legend to indicate shell integrity in terms of crystal thickness and density.

Table A1: Please include other calculated parameters such as HCO3-, CO32- and CT.

RESPONSE: Suggestion will be adopted.

References used for review:

Weiss et al (2002) Mollusc larval shell formation: amorphous calcium carbonate is a precursor phase for aragonite. DOI: 10.1002/jez.90004

Kristova et al (2015) The effect of the particle size on the fundamental vibrations of the [CO3(2-)] anion in calcite. DOI: 10.1021/acs.jpca.5b02942.