Biogeosciences Discuss.,
doi:10.5194/bg-2017-85-AC3, 2017

Biogeosciences Discussions
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EGU

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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Received and published: 31 May 2017

The manuscript " Changing mineralogical properties of shells may help minimize the impact of hypoxia-induced metabolic depression on calcification" by Y.S. Leung and K.M. Cheung describes physiological and shell-compositional responses of a calcifying polychaete to hypoxia and predator stress. The research question as such is original and could potentially provide interesting data to the community. However, the experimental setup and the analytical methods used are inappropriate, at least in the way they are described in the manuscript in the present form. The main points that

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need to be addressed:

- 1) stability of DO over the culturing period needs to be reported. Stability in DO could be impacted severely by a) gas-exchange with air (headspace/caps used), b) addition of photosynthetically active algae and c) addition of 1/3 of non treated (e.g. normal DO) seawater every 3 days due to feeding.

2) bubbling with N2 potentially changes also CO2- C-system parameters, also over time, need to be reported

If DO/ C-system were not stable over time, results could be negatively impacted. Furthermore, the data is not presented sufficiently, individual measurements of all parameters need to be reported in a table along with averaged values and standard deviation. These points render the manuscript unfortunately not suitable for publication in its current form. If the authors can address these points, the manuscript could be reconsidered for submission. I wish the author good luck with their resubmission and remain available for further feedback and discussions.

RESPONSE: We are sorry for excluding seawater data in the previous submission as we thought that they have limited interpretive values. Seawater data will be reported in the revision as requested. We appreciate reviewer's blessing for the resubmission.

Main points that need addressing:

-Were any other parameters of the C-system measured in combination with pH to assess stability over time? Bubbling with N2 not only strips oxygen but can also strip CO2. If the two treatments (hypoxia/normoxia) are compared, it needs to be ensured that the C-system was similar, otherwise the effects could be attributed to calcification response to changing C-system (higher carbonate ion conc. caused by higher pH) and not solely to hypoxia effects.

RESPONSE: This comment is also raised by Reviewer 1. We will add seawater data and discuss the potential effect of basification in the revision.

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-If I understood correctly, water was exchanged during the culturing period every 3 days. Was the bottle filled headspace free and sealed that air in the headspace on top of the bottle did not equilibrate with the air outside? Was DO conc. in the bottles measured after 3 days to measure stability in DO conc.? Also, the algae provided photosynthesize, potentially changing DO and pH, was this accounted for? If food was added each day a 20ml, after 3 days, 60ml out of the total of 180 ml (=1/3) does not stem from DO adjusted seawater, so DO concentrations (and pH) could have been significantly different after 3 days.

RESPONSE: The glass bottle was covered with a lid to prevent interaction with air outside. Two holes were drilled on the lid: one for inserting an airline to supply the gases continuously and one for equalizing the pressure inside and outside the bottle. This information will be added in the revision for clarity. DO concentration was monitored regularly throughout the 3-week exposure period. We need to emphasize that the effect of respiration and photosynthesis on DO concentration was negligible because the seawater in the experimental setup was continuously aerated so that stable equilibrium of gases can be achieved throughout the experiment (Ln 76-80). As such, the minimally increased DO concentration due to addition of algal suspension can be returned to the desired DO level rapidly (i.e. negative feedback).

-Shell growth- How was shell growth measured? Were the individuals Id-ed and growth measured over time or just at the end? Please report shell growth data. How many individuals were cultured? How were the parts of the tube that were added during treatment identified? I assume only parts added in treatment were chosen for analysis of shell properties or was the whole tube analyzed? If analyzing the whole tube, this could potentially mask shell effects on shell properties, as a certain amount of shell would stem from non-treatment conditions. Please also report individual data of shell property measurements.

RESPONSE: Shell growth was estimated by measuring tube length (Ln 95), while shell growth rate is given by (final tube length – initial tube length)/exposure time (Leung and

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Cheung, 2017). Ten individuals were cultured in each bottle (Ln 89). Microcentrifuge tubes were labelled to identify each individual (one tube, one individual). We measured tube length three times (before, in the mid of and after the exposure), but overall shell growth rate has the greatest interpretive value. The shell growth data can be provided as supplementary information. As for the analysis of shell properties, we only used newly-produced shells (Ln 96-97) because the properties of old shells are probably unchanged. It is very easy to identify the newly-produced shell. Photos will be provided in the revision for illustration.

-Was salinity monitored over the experimental duration? If the air/N2 mix while bubbling was not moist, this could cause salinity to change. Salinity changes also would cause changes in Mg/Ca in the seawater used for culturing, possible causing the Mg/Ca changes reported here (partly). Was Mg/Ca measured in the seawater? In what unit is Mg/Ca reported in Table 1? Mmol/mol?

RESPONSE: Salinity was checked regularly throughout as H. diramphus is relatively sensitive to salinity change. Salinity was very stable because seawater was renewed once every three days and seawater evaporation is minimal in the bottle with a lid. Unfortunately, we did not measure Mg/Ca of seawater, which has a stable value on a large geographic scale. Regardless, we used the same bulk of seawater across treatments so that no bias was induced. Mg/Ca is a molar ratio and we will add "(molar)" after "Mg/Ca in calcite" to match Ries's presentation, which is common in climate change research (e.g. Ries, 2010).

Minor points: -l. 68- preliminary study: Please provide data- how many organisms were studied, how was survival assessed, how long lasted culturing period, what were experimental conditions (food/temperature, salinity, etc..)?

RESPONSE: The method was described in our previous study (Leung et al., 2013), except that different species was used. After careful consideration, we decided to remove this redundant sentence as polychaetes are generally regarded to have strong

tolerance to hypoxia (Vaquer-Sunyer and Duarte, 2008). We will provide the survival rate following exposure to show the tolerance of H. diramphus to hypoxia. This can substantiate that H. diramphus is a suitable species for this study, while avoiding detailed description of the unpublished data in the preliminary study, which is not very relevant to this study.

-measurements of water parameters (l. 73)- what instruments were used? Salinity either unitless or use psu, what pH scale is reported?

RESPONSE: Information on instruments (e.g. pH meter, refractometer, etc.) used for each water parameter will be added in the revision. NBS scale was reported.

l. 133- please report blank values so the reader can assess, how much gas exchange through the syringe occurs over an hour.

RESPONSE: Suggestion will be adopted in the revision.

References

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2017-85, 2017.