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Biogeosciences Discussions

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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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

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.

With kind regards, Nina Keul

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.

-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.

-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.

-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?

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..)?

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

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

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