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Comment on bg-2021-34

Anonymous Referee #2

Referee comment on "Anthropogenic CO_2-mediated freshwater acidification limits survival, calcification, metabolism, and behaviour in stress-tolerant freshwater crustaceans" by Alex R. Quijada-Rodriguez et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-34-RC2, 2021

GENERAL COMMENTS

This is an interesting study that addresses an important gap in our knowledge, that of the physiological responses to elevated CO2 (which also causes acidification) in freshwater crustaceans rather than marine organisms that have been studied more often. The authors have used the Chinese mitten crab as a useful model organism for this and carried out experiments involving exposure to elevated CO2 for durations varying from 1 week to 6 weeks, measuring mortality, haemolymph acid-base balance, carapace calcium content and behaviour. The findings are potentially very useful, but as the authors need to provide more detail for some of the methods/approaches used. They should also consider potential alternative interpretations of their data based on the nature of some of the experimental methods used. I have provided a list of specific comments below in the hope they will be useful in improving the clarity and quality of the manuscript.

SPECIFIC COMMENTS

Whilst open ocean seawater is extremely consistent in its chemical composition (at least for a given salinity), freshwater is definitely not consistent, and in fact is extremely variable, in ways that can have major consequences for physiological responses to variables such as CO2/pH. It is therefore important to report details of the freshwater chemistry, more than just the carbonate chemistry in Table 1. In particular ion concentrations that are relevant for gill ion and acid-base regulation processes (e.g.

sodium and chloride), and calcium is critical for understanding and interpreting potential calcification effects of the treatments.

To help the reader the authors should provide a conversion, or direct comparison, for pCO2 values reported in Pa and uatm.

Some variables were measured over a 7 day exposure period (haemolymph acid-base, oxygen consumption rate and ammonia excretion rate), or 14 days (mortality), but others (calcification and behaviour) seem to be over 6 week exposure. These different timescales are not explained in Methods section, or justified.

What caused mortality? In particular could this have been related to cannibalism after individual crabs had moulted? This seems likely, as is common in crustaceans in aquaculture where animals have little chance to escape their conspecifics whilst waiting for their exoskeleton to harden after moulting. There is no mention of hides or shelters being provided in the exposure tanks, so if crabs were moulting it is possible that calcification was slower in the high CO2 treatment, resulting in more crabs being prone to cannibalism whilst waiting to calcify, rather than an inability to calcify eventually (given enough time). With 6-7 crabs in a 10 litre tank cannibalism seems likely if some were moulting.

Why not calculate the actual ammonia quotient (AQ) and include discussion of these data regarding protein utilisation, and reference the AQ values found in other species and how these numbers relate to protein utilisation.

In the locomotory behaviour tests (and metabolic rate and ammonia excretion rate measurements) it is important to report data for the carbonate chemistry variables

actually measured (at the same time) in both the experimental holding tanks the crabs were taken from, and the arena tanks the behaviour was assessed in (or respirometers). This is important because if they were different pCO2 values it could result in a rapid acid-base disturbance in the crabs transferred from one tank to another that could be the cause of behaviour differences or metabolic rate differences, rather than the actual prior high pCO2 exposure.

There is considerable discussion of the data showing a metabolic depression caused by freshwater acidification. However, if I understand the Methods accurately, metabolic rate (as oxygen consumption rate) was only measured for a single 30 minute period in each crab, and this was only after 15 minutes "acclimation" following handling and transfer to the respirometer chamber. If this is the case, then what was measured cannot be considered as the stable metabolic rate during exposure to either treatment (low or high CO2), and "metabolic depression" is not an accurate conclusion to make. Instead, what was measured is more likely to be the acute metabolic response to handling, brief air-exposure and transfer to a new environment, on top of the effects of any prior exposure to the CO2 levels used. This has not been considered but is important in interpreting the data reported.

The manuscript often refers to "calcification" being measured, but this implies the rate of calcification which was not actually measured. Instead, carapace calcium content was measured at a few timepoints, which has been used to imply "calcification rate", but that is not strictly true. See also comments above about moulting, immediately after which is when the greatest rates of calcification occur.

L.77-78 – The description of how pH/CO2 was controlled is not sufficiently detailed to provide a full explanation. Presumably this was done using 4 pH electrodes permanently recording the pH in each of the 4 individual experimental tanks, and the signals received from each electrode by 4 separate pH controllers was used to regulate the flow of CO2 via air stones into these individual tanks? Please provide enough details to clarify this issue.

L.79-80: "Following injection of CO2 to regulate water pCO2 we recorded a brief pCO2

overshoot to a maximum level of 570Pa resulting from direct CO2 injection into the experimental tanks by the pH controller."

The water chemistry data for pCO2 in the table is reported in uatm, whereas here in the text it is given in Pa. This is confusing for most readers so I suggest the authors stick to one set of units, or provide both for direct comparison.

l.86 – Given that the CO2sys program requires salinity as an input variable to calculate carbonate chemistry, how was salinity measured, and what value(s) were used in these calculations?

Table 1 – It is not clear what these data are reporting, i.e. what timepoints do these data represent? From which experiments (the 7 day, 14 day or 6 week experiments?), and how were the means calculated with respect to the four different replicate tanks per treatment? More details are needed. It would seem appropriate to report data separately for the different duration experiments (7 day, 14 day or 6 week).

l.115 – How were the crabs selected "randomly"? Unless a truly random method was used, this usually means the first animals that were able to be caught be experiments, which can result in a bias based on behavioural traits of the animals.

l.119 – All units should be separated from their number by a space. So the 200mL should be 200 mL. This comment also applies throughout the whole manuscript.

The control values for haemolymph pH are very high (pH >8.1) for an aquatic animal at the temperature used (23 degrees C). The haemolymph bicarbonate is also surprisingly high (13-14 mM) in the control conditions (time zero for both treatments). Studies on other crustaceans suggest haemolymph pH at this temperature would be closer to 7.6-7.8 and bicarbonate closer to 3-6 mM, and usually only reach values this high if the animals were already exposed to very high CO2 (e.g. >10,000 uatm) and had accumulated bicarbonate to compensate pH. Perhaps there is a precedent for such high bicarbonate and pH in this species, or other crab species, that I am not aware of, but the authors provide no discussion or comment on this discrepancy.