

**Subject: Comment on bg-2021-34**

**Authors' responses are in italics**

**Reviewer #1**

**1. Length of experiment-** In the methods section, the authors need to state the duration of their experiments. I noticed on the results table that for some measurements, the time axis extended to 8 weeks while in others 7 days, so I'm assuming that different experiments were done for different lengths of time? If so this needs to be explicitly stated somewhere in the methods.

*In the methods section we will clearly state the timeline of exposure for each measured parameter.*

**2. Line 37: "To date there are no comprehensive studies investigating various physiological and behavioral effects of realistic future levels of CO<sub>2</sub>- mediated acidification in calcifying freshwater invertebrates".** This is false. David et al. (2020) investigated the effects of CO<sub>2</sub>-induced acidification on the invasive freshwater gastropod, *Viviparus georgianus* over a 12 week period (Journal of Molluscan Studies 86:259-262). They used shell repair as a proxy for physiological performance. The findings of that study was the first to assess the effects of CO<sub>2</sub>-induced acidification in a calcifying freshwater invertebrate and regardless of whether the authors think it was 'comprehensive' or not, that reference should still be somewhere in the manuscript and the results compared with theirs.

*We respectfully disagree with the reviewer on the inclusion of David et al. 2020 study within our manuscript. We would like to point out that we stand by the statement that there are no **comprehensive** studies that use **realistic future** CO<sub>2</sub> levels. While other studies have investigated elevated CO<sub>2</sub> in freshwater animals the CO<sub>2</sub> levels employed are typically far beyond what could realistically be expected for near future FW acidification. In the David et al. 2020 study mentioned by the reviewer the effects of CO<sub>2</sub> induced acidification on shell growth rates of a freshwater gastropod were measured. In that study, the acidification treatments were based on a CO<sub>2</sub> induced pH drop from pH 7.3 to pH 6.8 and 6.3. Unfortunately, this manuscript does not provide key water parameter data (water alkalinity, water total carbon or water pCO<sub>2</sub> levels) that allows any reader to accurately quantify whether the treatments used are within a range that could realistically be seen in future FW systems. In addition, since the treatment pCO<sub>2</sub> levels are unknown and sufficient data has not been provided for the reader to make the calculation themselves it is difficult to then take this study and make a direct comparison to our study as it is crucial in making comparisons between freshwater/ocean acidification studies to know the CO<sub>2</sub> levels employed.*

3. What was the rationale for using the Chinese mitten crab for this study? No information is provided on the study species in terms of life history, fecundity, etc? The authors mentioned that it is a model organism for studying climate change but do not actually explain why.

*We will add more information on the reasoning for using Chinese mitten crab in the manuscript. We do not state it is a model organism for studying climate change but state that it could be an interesting model as this species is an invasive species that is quite tolerant to a range of environmental parameters.*

4. Very little is mentioned on the invasive status of Chinese mitten crabs in other parts of the world and nothing is mentioned about the implications of the findings of this study on the management/control of invasive populations of the species. This is what I meant when I mentioned earlier that the authors did not put their findings into a broader context.

*As mentioned above we will add a bit more about our reasoning for using Chinese mitten crab and their invasive status/importance in aquaculture in Asia. In terms of trying to extend our results into the implications of this study on management and control of invasive populations we would more likely want to refrain from making too bold of claims as this is really a first probing study into the effects of freshwater acidification and our results here do not necessarily translate into how future populations will actually be affected as we are not considering multiple factors such as generational adaptation to changing environments. We would like to try and avoid over-reaching the implications of this study into say management and control of invasive species as we believe an initial probing study shouldn't be trying to make that bold of a claim but more provide the grounds for further studies that can build on our findings.*

5. **Line 65:** The authors mentioned that these crabs were 'purchased'; does that mean that they were bred in a controlled setting prior to arrival? If so, how sure are you that their physiological responses are reflective of what happens in natural populations?

*Yes, the crabs were purchased. However, our source wild catches their crabs and then rears them to adulthood. Since we were purchasing juvenile crabs they would have been early wild caught crabs and were then maintained under lab conditions for several weeks prior to experiments. This will be clarified in the manuscript as they are not traditionally aquaculture bred and reared crabs but derived from wild stocks.*

6. **Line 71:** Is oatmeal and mollusc meat the type of food the crabs would usually eat in their natural environment? Is that a best practice for doing controlled experiments on crabs in the lab? (If so please provide reference).

*Chinese mitten crab are opportunistic omnivores that in early life mainly eat plant material but become more carnivorous as they grow. Studies have essentially shown they eat plant material, small invertebrates (including bivalves), and injured/dying/dead fish. We chose oatmeal due to personal communication for our aquaculture source that this is something they do feed the crabs. We will add a citation of a report from the department of fisheries and oceans Canada supporting this dietary behaviour.*

7. If you collected juveniles and cultured them under these stressors, why not measure the growth rate? In fact, why wasn't size measurements taken prior to each experiment?

*Unfortunately, due to logistic reasons we were not able to fit a growth experiment into this study. The vast majority of our experiments were not done over an extended period of time and as crustaceans must molt to grow the length of our experiment would unlikely allow for an accurate analysis of growth rates. We did perform the carapace calcium content experiment over 6 weeks but as we were periodically sampling, we would have only had a sample size of roughly 8 crabs that made it to the 6 week point which we feel would be too small of a sample size for proper growth experiments.*

8. What was the sample size for each experiment. This needs to be included either in the methods or when reporting statistics in the results section.

*Sample size for each experiment is present in the figure captions but can be written into the text if necessary.*