

General. This paper reports results from experimental exposure of a freshwater crab to elevated CO₂ levels. The authors develop a justification based largely on the paucity of prior studies of biological CO₂ effects of freshwater taxa. The range of response variables is large and impressive including metabolic rates, a battery of physiological metrics, locomotor behavior, and survival. In total, the test organism was found to be CO₂-sensitive in most of the responses measured which to actually runs counter to the authors' expectation for this taxon which happens to live in CO₂-variable habitats (more on this below). The paper provided a good summation of the literature and was reasonably well written (corrections and suggestions are identified below under 'Technical corrections').

Specific comments. I had two issues with the paper. The first is related to the packaging and broader context for the study. The second issue pertains to the analysis of the data.

Regarding the packaging of the study, the authors can make the paper clearer by lessening the effort to cover all bases as they describe the motivation and summary of their work. They begin this journey in the Introduction by providing context and underscoring the lack of studies on the effects of CO₂-induced acidification in freshwater taxa, especially caldifiers, relative to their marine counterparts. Fine, but one paper will not significantly change that balance. In fact, shortly after making this case, the authors add a 'but wait' because the Chinese mitten crab might not be sensitive to elevated CO₂ due to its occurrence in freshwater habitats that range widely in CO₂ concentrations. So it indeed might not be a representative freshwater taxon to study CO₂ effects after all. The reselling of mitten crab (actually, selling the paper about mitten crab) picks up again in the Discussion but from a different, all things to all people, vantage "we aimed to demonstrate for the first time the physiological and behavioural consequences of a possible future CO₂ mediated freshwater acidification scenario on a juvenile calcifying invertebrate, the Chinese mitten crab." I would much prefer that the authors provide a more modest and clear appraisal of the import of their work and their working hypotheses, and let the readers assign value to their efforts.

The second issue is more problematic as it pertains to the experimental design (including response variables) and data analyses. The following points are interrelated and would benefit from a synthetic solution. One is suggested below.

1. It is unclear to me why the time-course data were run as a one-factor design (one-way ANOVA) rather than a two-factor design as was done for the other analyses. It would seem that a two-way analysis is appropriate and would provide the same hypothesis test as the one-way plus more (factor 1 vs factor 2 and test for the interaction between factors 1 and 2). Regarding the presentation of results in Table 1 from the series of one-way ANOVA's, the 'Treatment' appears to be incorrect as the control FW or the acidified FW treatment was only provided as one 'type' (i.e., the crabs were exposed to control FW in the 'Control FW' treatment OR were exposed to acidified FW in the 'Acidified FW' treatment, and not different levels of each factor) so what was being tested? Was it the change in response over time? If so, in this case 'Time' would be the 'Treatment' (aka factor) and Control FW or Acidified FW was the study condition. If I am interpreting the table correctly, the table needs to be restructured as does the language describing the tests.
2. Building on #1, it seems that many of the data types are based on repeated measures over time. Hence, a repeated measures or profile analysis would seem appropriate.
3. Building on #1 and #2, the response data collected in the experiments are highly interrelated. Within an experiment (e.g., locomotor behavior) the responses are clearly interdependent as well as repeated. Further, the authors make this very point in the Discussion, i.e., the responses are interrelated. For example, locomotion and energy expenditure in general are more labile in order possibly to conserve other responses more aligned with survival (Lines 403-04). There is likely a degree of nesting or hierarchy of responses (e.g., the locomotor behavior metrics are

more tightly interrelated responses within the entire set of responses used) but the authors DO argue in the Discussion that there is likely a network of inter-relatedness or covariance among the responses. If true (it likely is), then employing a set of univariate tests (i.e., the one-way and two-way univariate ANOVA's) is the wrong approach.

I would recommend revamping the statistical analyses. Run the data as two-way multivariate analyses (either MANOVA's and/or repeated measure designs). Discount the critical p -values to accommodate multiple tests on the same set of data (i.e., data on the same individuals in some cases or crabs drawn from the same tanks).

Technical corrections. Below are examples where corrections or reconsiderations are needed.

1. Be explicit when referencing the data, e.g., identify the response variables rather than refer to 'time-course data' (Line 160) as it would seem that multiple data types were recorded over time.
2. 'Data' is plural subject, e.g., data were.
3. Run spell check as there were misspellings (e.g., Line 405, 'consequences')
4. Hyphens for compound adjectives (e.g., Line 129, 'closed-system respirometry') were commonly omitted
5. Inconsistencies in nomenclature after establishing a convention, e.g., SEM (Line 174) vs SE (Figure 1 legend).
6. Regarding the Conclusions, avoid closing with a wish list with little, if any, connection to prior text or the themes of this paper (e.g., Lines 418–420), "Future biological studies should emphasize transgenerational adaptability" – yes but seems out of place here. "... long-term effects of freshwater acidification on the scale of weeks to months" – allude to this theme earlier in ms. "...assessment of a wide range of freshwater species to determine animal performance indicators for CO₂ sensitive species" – this is the one of the three in this wish list that has a direct connection to this paper.