

Author response to comments by Anonymous Reviewer #2

*RE: Review of the MS entitled 'Physiological compensation for environmental acidification is limited in the deep-sea urchin *Strongylocentrotus fragilis*' by Taylor et al. Biogeosciences Discuss. 10, 8313–8341 [doi:10.5194/bgd-10-8313-2013].*

*Taylor and colleagues have characterised the acid-base and life-history traits responses of the deep-sea urchin *Strongylocentrotus fragilis* exposed to elevated pCO<sub>2</sub> and lower pO<sub>2</sub>. The authors have conducted two experiments: i) a 31 days exposure at the end of which they define the coelomic fluid acid-base status of the urchins, ii) a 140 days exposure at the end of which they determined urchins locomotion, feeding, growth, and gonadosomatic index. The authors then use this information to infer on the bathymetric distribution of the study species in predicted future conditions. The experimental work seems solid and accurate, and the MS present novel and very interesting data on the responses to elevated pCO<sub>2</sub> in a deep-sea species of urchins already living at low pO<sub>2</sub> conditions. The work is original and worth publishing but not in its current form. In general the MS needs major re-writing in some areas, in order to improve in structure and clarity.*

*Major issues to be addressed:*

*i) The Introduction needs to be more focused and to gain a better flow. The text need some restructuring and rewriting to help the story you tell in your MS to better emerge. The passages from a sentence to the next are often abrupt, and at times it is difficult to work out the link between sentences.*

**Author reply:** Thank you for this insight; we have gone through the introduction (and the manuscript as a whole) and edited the text throughout for clarity.

*ii) The Introduction needs a clear Aim before you introduce the study species. It is commendable you provide a hypothesis, but an Hp has to generate from a rational and an aim.*

**Author reply:** We have gone through the introduction and edited the text throughout to clarify the aim of the study before introducing the study species; in particular, a sentence has been added to the end of introduction paragraph one to clearly introduce our aim.

*iii) In the Methods section you state that <<No animals were fed during the experimental period>>. First 31 days of starvation appear excessive if I understand correctly what the authors are saying; second, starving can dramatically change the metabolic status, and thus potentially the acidbase status of the sea urchins. This needs at least to be thoroughly discussed, and limitations of the interpretation of the data should be at the very least recognised.*

**Author reply:** We apologize for this oversight; animals in experiment one were indeed fed every approx. seven days, but no sooner than 72 hours before extracellular fluid sampling. We have edited the text to correctly reflect our methodology.

*iv) The statistical analyses can be improved by adding a covariate (use as appropriate the initial or final size of sea urchins), effectively transforming your ANOVAs in ANCOVAs and thus utilising some 'individual' information which should increase the test power in discriminating differences,*

**Author reply:** Thank you for bringing this to our attention; we have rerun the appropriate statistical tests as suggested, and detailed these methods and results in the edited text.

*v) More on the statistical analyses: as you find that in experiment two most data did not meet assumptions of normality, did you try any transformations before to pass to non-parametric tests? Even if transformation were not beneficial you may have anyway a sufficient number of treatments with a sufficient replication to assume ANOVA/ANCOVA test are resilient enough when assumptions are not met/fully met (see Sokal and Rohlf).<sup>22</sup> Finally, you should employ a post hoc test to test for differences among treatments beyond the ANOVA test? A t-test (unless you use a Bonferroni correction) is not an appropriate test, as it is not sufficiently conservative. Tukey test of Dunnett test or similar ones are more appropriate.*

**Author reply:** We have rerun the appropriate statistical tests as suggested, using ANOVAs followed by Tukey's test for pairwise comparisons. In cases (see experiment two) where normality assumptions were not met even after multiple types of data transformation, Kruskal Wallis ANOVAs were used and followed by Mann-Whitney U-tests for pairwise comparisons. These changes are described in the edited text.

*vi) The results on the acid-base status need to be fully shown in the MS (see below for details),*

**Author reply:** We have addressed the below (detailed) comments and now include the full results of acid-base status.

*vii) The Discussion make some good points, and the argument for the potential shift in bathymetric range shift/restrictions are interesting. However, the Discussion is also very brief and before you get to infer on the potential ecological consequences of the data you should further develop (expanding, going in more depth) the argumentation around your own data in comparison to (for example) those on the responses of other deep-sea species and/or sea urchins from other habitats to elevated pCO<sub>2</sub>.*

**Author reply:** We have edited the discussion text to further develop the argumentation around our own data in comparison to those on the responses of other deep-sea species and/or sea urchins from other habitats to elevated  $p\text{CO}_2$ .

*Detailed comments:*

- Page 8314 line 9: change 'internal acid-base balance' to 'extracellular OR coelomic acid-base status' throughout the MS.

**Author reply:** We have edited the text throughout to consistently use "perivisceral coelomic fluid (PCF)".

- Page 8315 line 18: 'Pörtner' not 'Portner'.

**Author reply:** Apologies- this is spelled correctly in our original MS and we will review future proofs more closely.

- Page 8315 line 24-25: it is unclear what you mean with 'more phylogenetically derived animals'.

**Author reply:** We have edited the text to clarify we are referring to more phylogenetically "advanced" taxa

- Page 8315 line 25-27: please rephrase the sentence 'Studies have shown major species-dependence in the acid-base regulatory capacity of sea urchins'.

**Author reply:** We have rephrased this sentence for clarity

- Page 8316: line if you have thoroughly verified the literature and you are positive there is no other studies which have characterise the acid-base status of deep-sea urchins, you can remove 'To our knowledge'.

**Author reply:** We have re-evaluated the literature and removed "to our knowledge" from this sentence.

- Page 8316 line 18-23: please reintegrate this section above where you introduce the effects of OA.

**Author reply:** Sections have been integrated as suggested.

- Page 8316 line 24-Page 8317 line 4: this section should be removed completely as it confuses the reader about what your article is going to talk about. Your work does not address experimentally the effect of  $p\text{CO}_2$  on an ecosystem functions and thus to find this argument in the Introduction it only confusion. Comments on this aspect could be made in the Discussion.

**Author reply:** The section referenced has been completely removed to avoid lengthy discussion of peripheral topics.

- Page 8317 line 15-21: *define the duration of each experiment here.*

**Author reply:** Text has been edited as suggested to provide the duration of each experiment.

- Page 8318 line 1: *change 'animals' to individuals' here and throughout the text when you mean individuals (e.g. the use of the term animals is correct in the Introduction).*

**Author reply:** We have edited the text throughout to use “individuals” rather than “animals”.

- Page 8318 line 5: *salinity does not have an international recognised unit of measure. Please remove 'ppt', just state 'salinity 34'.*

**Author reply:** Text has been edited accordingly.

- Page 8318 line 6: *section 2.2, this section is more 'experimental set up and procedure' than 'experimental design' (section 2.3 and 2.4 are exp. design).*

**Author reply:** Section 2.2 has been renamed 'Experimental setup and procedure'

- Page 8318 line 20 and page 8320 line 11: *should CCO<sub>2</sub> here be DIC? Please check the EPOCA guidelines for the best use of the term CCO<sub>2</sub> and DIC.*

**Author reply:** Text has been edited accordingly to refer to total CO<sub>2</sub> as DIC in reference to seawater chemistry, and as C<sub>CO<sub>2</sub></sub> in reference to coelomic fluid chemistry (per 2010 Guide for Best Practices)

- Page 8319 line 16: *change 'over 31 days' to 'after 31 days', same where it applies throughout the MS.*

**Author reply:** Text has been edited accordingly.

- Page 8319 line 21: *change 'per' to 'per' and for all Latin forms across the text make sure there are italicised (incl. in situ, via).*

**Author reply:** Text has been edited accordingly.

-Page 8319 line 21: *it would be best to define the treatments as pCO<sub>2</sub> rather than pH, and O<sub>2</sub> concentration as partial pressure (pO<sub>2</sub>).*

**Author reply:** We have edited the text to define the treatments as “control, moderate, high, and extremely elevated pCO<sub>2</sub>”, and included Table 1 to more

precisely define the treatment chemistry for experiments one and two. Since the  $p\text{CO}_2$  values are not simple/round numbers, we think referring to these  $p\text{CO}_2$  treatment designations in addition to pH is the best solution for the reader.

- Page 8320 line 14: please provide the full version of the Henderson–Hasselbach equation.

**Author reply:** Text has been edited to include the full rearrangement of the Henderson-Hasselbalch equation.

- Page 8320 line 19: when you give a number (not associated to a unit of measure) which is less than 10, you should write it in letters rather than numbers (as in 'six days' instead of '6 days').

**Author reply:** Text has been edited accordingly.

- Page 8322, sections 2.4.1 to 2.4.4: if available please provide reference to standard methods. For example in line 15 you say << A validated blotting technique was used to ensure consistency in attaining kelp weights on removal from treatment jars.>>, but do not say if it is validate by you and how, or it is from a peer-reviewed article.

**Author reply:** Text has been edited accordingly to clarify the 'blotting' methods in question.

- Page 8323 section 2.5: it needs more details. You must provide (even if synthetically) details on the statistics values, df, and p-value for normality and equal variance tests determined by the Shapiro–Wilk test and the F Test for equal variance.

**Author reply:** We have edited the text to include appropriate statistical details.

- Page 8324 Results section: the results for the acid-base status are not properly report. As you measure coelomic fluid  $\text{CO}_2$  and pH you should at least report the mean and SE/SD/95%CI of these two parameters, although I find it is good practice to report means and SE/SD/95%CI for the parameter you derived from the Henderson–Hasselbach equation (i.e.  $p\text{CO}_2$  and  $\text{HCO}_3$ ). Please report these parameters in figures or a table, with the relative statistics. Whilst the Davenport diagrams are important tools to represent and help discussing the acid-base status of a study organism, they are an elaboration on the data you collect, and data you derive from a calculation, thus first you must provide the 'true' data and then its representation. Notice that some authors provide the Davenport diagrams only in the Discussion, with the rationale that they are not strictly speaking a result but a subsequent elaboration. Furthermore, in the Results you state <<As shown in pH-bicarbonate (Davenport) diagrams (Fig. 4), a pattern of significant (ANOVA,  $F=9.68$ ,  $30.46$ , and  $30.55$  for pH 7.5, 7.1, and 6.7, respectively;  $p < 0.0001$  in all cases) hypercapnic-induced acidosis persists in *S. fragilis*.>>. How can you statically test for a multidimensional representation of the data (please note there are effectively three axes: coelomic pH,  $\text{HCO}_3$ ,  $p\text{CO}_2$ ) with a

*single ANOVA test? You should provide statistics for all four parameters (i.e.  $\text{CCO}_2$ , pH,  $p\text{CO}_2$ ,  $\text{HCO}_3^-$ ).*

**Author reply:** We have simplified our reporting of these results by adding a figure (new Fig. 2) showing trends for each of the four parameters (i.e.  $\text{CCO}_2$ , pH,  $p\text{CO}_2$ ,  $\text{HCO}_3^-$ ) at each treatment level. In reporting these data as a modified figure, we have elected to exclude our previous Davenport diagram figure, so as to not burden the reader with needless repetition of data/ results.

*- Page 8325 line 4: 'flip time' is best defining as 'righting up time', this is important because this is the term which has been used in many works before and thus would help those seeking this information more easily accessible.*

**Author reply:** Thank you for bringing this to our attention; we have edited our text to refer to "righting time", as seems most commonly used in the literature.

*- Discussion section: unless strictly necessary (e.g. it is the first time you refer to a figure) it is not good practice to refer to the figures (which is something you do in the Results section only).*

**Author reply:** Thank you; we have edited the MS throughout to reflect this suggestion.

*- Merge Figure 1 and 2 together.*

**Author reply:** Ok.

*- Remove Figure 3.*

**Author reply:** Ok.

*- Introduce a figure with the acid-base status parameters and relative statistics.*

**Author reply:** Ok.

*- Figure 4 should be the last figure.*

**Author reply:** Since we now report these data as a modified figure, we have elected to exclude our previous Davenport diagram figure, so as to not burden the reader with needless repetition of data/ results.