Author reply to comments by Anonymous Referee #1

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Taylor et al. present the first long - term acclimation study of a deep - sea calcifying invertebrate to the combined impacts of ocean acidification and hypoxia. This is an important area of research that has been neglected so far. The study has been carried out well and gives valuable insights into the ecophysiology of the sea urchin S. fragilis. The discussion would benefit from a stronger focus on expected scenarios of change and habitat variability. The paper will be a very good contribution to the field following careful revision.

Author reply: Thank you for these thoughtful and thorough comments. We have edited the discussion (especially the second to last paragraph) to focus more on expected scenarios of change as well as adaptation capacity as described in later reviewer comments. After taking into account the suggestions of all three thoughtful and thorough reviews, we find our manuscript to be much improved, and hope you agree it will be a useful contribution to the BG literature on ocean acidification.

General comments:

1.) A table with information on carbonate chemistry is missing. Treatment levels (PCO2) should be given in addition to pH values. PCO2 is the relevant variable that impacts extracellular acid-base status in this and many other marine invertebrates.

Author reply: Thank you for bringing this oversight to our attention; we have added a table (Table 1) showing carbonate chemistry and show PCO_2 and DIC in addition to pH.

2.) Statistics: use non - parametric alternatives to ANOVA (e.g. KW test) instead of Utests, use Posthoc tests and repeated measures ANOVA where indicated.

Author reply: As *per* another comment by the second reviewer, we have re-evaluated our data using transformations as described in the edited text. pH data became normally distributed when converted to H^+; thus, ANOVAs with Posthoc tests are now described in the edited text. Other data that were not made normal by transformation have been re-evaluated using KW ANOVA followed by appropriate Mann-Whitney pairwise tests. A Bonferroni correction was applied to these p-values as described in the edited text.

Specific points:

P8314 L18 I would be more careful here with such statements, see e.g. Dupont et al. 2012 Mar Biol for strong impacts of longer-term acclimation (>1 year) or Miller et al. 2012 Nat Clim Change for impacts of trans-generational acclimation, or Lohbeck et al. 2012 Nat Geosci for effects of multi - generation adaptation to OA. Also take a look at

Pespeni et al. 2013 PNAS, Sunday et al. 2011 Plos One (maybe add a brief section on adaptation capacity to the discussion).

Author reply: Thank you for bringing these studies to our attention; we have re-phrased to soften this idea in the abstract and discussion, and have included a brief discussion on adaptation capacity as suggested, including some of the above references (second to last discussion paragraph)

P8315 L23 Use either pHi or pHe.

Author reply: We have edited the text to clarify pHi vs pHe.

P8315 L27 Distinguish between dissolution of carbonates that cannot escape the extracellular fluids of invertebrates during tidal emersion and active bicarbonate accumulation (Spicer et al. 1988; see discussion in Holtmann et al. 2013 Mar Biol online early).

Author reply: We have edited the text to distinguish between test carbonate dissolution and active bicarbonate accumulation, citing the suggested references.

P8316 L11 Use more technical language.

Author reply: We have edited this section accordingly.

P8316 L16 Take a look at the literature, there are several examples that have investigated T and CO2 simultaneously, several that have looked at CO2 and O2 (see work of Burnett and coworkers).

Author reply: We have edited this sentence (and included additional references) to clarify that there are indeed examples of multiple OA stressors in the literature.

P8316 L23 Cite primary literature; I think that there are very few cases that clearly demonstrated shell carbonate buffering of extracellular pH.

Author reply: Indeed, there are very few studies that have quantified or even clearly shown shell carbonate buffering of pHe. We have revisited this topic in the literature and edited the text to reflect this, also adding primary literature.

P8317 L5 Intro in general: I would try to highlight potential links between acid-base status and energy budget allocation decisions (see e.g. Stumpp et al. 2012 PNAS, Aquat Toxicol). Also, it is probably worth mentioning somewhere that calcification initially is an intracellular process that must interact with cellular pH regulation processes (Beniash et al. 1997, 1999 Proc Roy Soc B). Habitat variability and future changes in these could also be better discussed (see e.g. calculated profiles in Brewer & Peltzer 2009 Science).

Author reply: We have edited the text to reflect these good suggestions, and now include the suggested references.

P8319 L15 Give info on sampling scheme for incubation water carbonate chemistry. Use the same units for seawater PCO2 and extracellular PCO2, so one can compare the diffusion gradients between both compartments. Why was such a high PCO2 (pH 6.6 treatment) chosen?

Author reply: We have edited the text to include more detail on the sampling scheme, and edited throughout to resolve the PCO2 units, in addition to adding Table 1 showing water chemistry measurements and calculations. The highest PCO2 treatment (pH 6.6) was chosen to clarify which processes would be the first and most severely impacted by elevated PCO2. Having this high PCO2 treatment not only added an additional data point for the effects we measured in other treatments, but also shed light on processes that were impacted more slightly (but significantly) in other treatments and thus may have slipped under the radar without the more dramatic change seen in the highest PCO2 treatment (for example, growth parameters). The text (intro) has been edited to briefly describe this advantage of including the highest PCO2 treatment.

P8320 L11 Terminology: I would use the same abbreviation for total CO2 (CCO2) and dissolved inorganic carbon (DIC or CT) to avoid confusion.

Author reply: Text has been edited to consistently refer to total CO2 as DIC in reference to seawater chemistry, and as C_{CO2} in reference to coelomic fluid chemistry (*per* 2010 Guide for Best Practices)

P8320 L16 The PCO2 isobars in the Davenport diagram do not seem to be correct. Recalculate - they should look similar to those in Pane & Barry 2007 MEPS or Stumpp et al. 2012 Aquat Toxicol, with strong increases in slope from low to high bicarbonate.

Author reply: At the suggestion of other reviewer comments, we have made changes to the way these data are presented; the new version does not include PCO2 isobars

P8321 L17 I am assuming that the system is a flow through experimental system, with water being discarded after passage through the experimental containers?

Author reply: Correct, our experimental is flow-through, with water discarded after passing through the experimental chambers. Text has been re-worded to clarify this setup.

P8324 L4 Give table with water carbonate chemistry.

Author reply: As per earlier comment, we have added the suggested table (Table 1).

P8321 L6 The authors should also discuss changes in extracellular PCO2 vs. seawater PCO2 in order to elaborate, whether diffusion gradients were maintained during hypercapnia acclimation. It appears that in treatments 2, 3 and 4, there is some accumulation of bicarbonate above control levels. Was this significant?

Author reply: Thank you for encouraging us to draw attention to diffusion gradients; in addition to adding details via the new Figure 2, we have edited the text to clarify where the changes in PCF [HCO3-] are significant and included a brief discussion of possible explanation for this; we have also and included further discussion of changes in PCF PCO2 vs. seawater PCO2 in order to elaborate, whether diffusion gradients were maintained during hypercapnia acclimation.

P8325 L4 The authors should use repeated measures ANOVA and appropriate Posthoc tests. Maybe talk to a statistician.

Author reply: As per another comment by Reviewer #2, we have revisited these statistical calculations using RM ANOVA with posthoc tests

P8325 L25 Reduction in size of sea urchin skeletons seems peculiar to me. Has this been shown before? What would the mechanism be? What is the accuracy of the image analysis of size? What about changes in body mass?

Author reply: We also found this result surprising; we find no reports in the literature of this type of response (i.e., shrinking in height) of echinoderms to elevated PCO2; however, the calcium carbonate matrix comprising the Strongylocentrotus exoskeleton lends itself to the fluctuations in size revealed by our study. We are confident in our sizing measurements but were unfortunately unable to measure body mass before the experiment (S. fragilis is particularly susceptible to releasing extracellular fluids through the Aristotle's lantern when removed from water). We have edited the discussion to reflect these observations.

P8326 L22 I disagree; you measured significant accumulation of 1-3 mM bicarbonate C3372 in 3 of the high PCO2 groups. The results might look different once the PCO2 isobars are corrected in the Davenport diagram.

Author reply: As per Reviewer #2's comment, we have added Figure 2 to more accurately detail our results of extracellular fluid acid-base balance. With the addition of this figure, we have eliminated the Davenport diagrams to prevent burdening the reader with excessive repetition of data/ results. The new Figure 2 shows the statistical details/significance of bicarbonate accumulation in the high pCO_2 groups.

P8327 L2 Stumpp et al. 2012 found 2-3 mM accumulation of bicarbonate above control levels and partial or full compensation of pHe. See Holtmann et al. for a detailed discussion and comparison with other studies.

Author reply: We have re-worded this section to more accurately reflect the comparison between previous results and those from our study.

P8327 L8 Fig. 1: where is the data from? Do you have PCO2 data? Any information on temporal variability in abiotic parameters in the habitat?

Author reply: The data used to construct Fig. 1 have been compiled by Jim Barry, and were collected from WOCE station P17C (central CA coast). The figure caption has been edited to reflect this data source.

P8327 L19 droebachiensis

Author reply: Thank you- this is correct in our original version and will be checked more closely in future proofs!

P8328 L9 Maybe mention that species from this genus can reach very high ages >50 years, hence a 'conservation' of adult health must be more important for lifetime reproductive success than loss of a single spawning season. Gonads are also known to be storage organs (Russell 1998 JEMBE, Spirlet et al. 2000 Aquaculture for gonad plasticity under culture conditions). Maybe also take a look at this paper: Mosch, Thomas, Sommer, Stefan, Dengler, Marcus, Noffke, Anna, Bohlen, Lisa, Pfannkuche, Olaf, Liebetrau, Volker and Wallmann, Klaus J. G. (2012) Factors influencing the distribution of epibenthic megafauna across the Peruvian oxygen minimum zone Deep-Sea Research Part I-Oceanographic Research Papers, 68 . pp. 123-135. DOI 10.1016/j.dsr.2012.04.014.55

Author reply: We have edited the text to include the suggested information, taking into account these and other references.