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## ***Interactive comment on “Physiological compensation for environmental acidification is limited in the deep-sea urchin *Strongylocentrotus fragilis*” by J. R. Taylor et al.***

### **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.

General comments:

1.) A table with information on carbonate chemistry is missing. Treatment levels

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(PCO<sub>2</sub>) should be given in addition to pH values. PCO<sub>2</sub> is the relevant variable that impacts extracellular acid-base status in this and many other marine invertebrates. 2.) Statistics: use non - parametric alternatives to ANOVA (e.g. KW test) instead of U-tests, use Posthoc tests and repeated measures ANOVA where indicated.

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).

P8315 L23 Use either pHi or 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).

P8316 L11 Use more technical language.

P8316 L16 Take a look at the literature, there are several examples that have investigated T and CO<sub>2</sub> simultaneously, several that have looked at CO<sub>2</sub> and O<sub>2</sub> (see work of Burnett and coworkers).

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

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

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(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).

P8319 L15 Give infos on sampling scheme for incubation water carbonate chemistry. Use the same units for sea water PCO<sub>2</sub> and extracellular PCO<sub>2</sub>, so one can compare the diffusion gradients between both compartments. Why was such a high PCO<sub>2</sub> (pH 6.6 treatment) chosen?

P8320 L11 Terminology: I would use the same abbreviation for total CO<sub>2</sub> (CCO<sub>2</sub>) and dissolved inorganic carbon (DIC or CT) to avoid confusion.

P8320 L16 The PCO<sub>2</sub> isobars in the Davenport diagram do not seem to be correct. Recalculate - they should look similar to those in Pane & Barry 2007 MEPS or Stumpff et al. 2012 Aquat Toxicol, with strong increases in slope from low to high bicarbonate.

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

P8324 L4 Give table with water carbonate chemistry.

P8321 L6 The authors should also discuss changes in extracellular PCO<sub>2</sub> vs. sea-water PCO<sub>2</sub> 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?

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

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?

P8326 L22 I disagree; you measured significant accumulation of 1-3 mM bicarbonate

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in 3 of the high PCO<sub>2</sub> groups. The results might look different once the PCO<sub>2</sub> isobars are corrected in the Davenport diagram.

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.

P8327 L8 Fig. 1: where is the data from? Do you have PCO<sub>2</sub> data? Any information on temporal variability in abiotic parameters in the habitat?

P8327 L19 droebachiensis

P8328 L9 Maybe mention that species from this genus can reach very high ages >50 years, hence '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.

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**BGD**

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