

Interactive comment on “Calcification, a physiological process to be considered in the context of the whole organism” by H. S. Findlay et al.

Anonymous Referee #1

Received and published: 26 April 2009

The manuscript submitted by Findlay et al. discusses the sensitivity of calcification processes in invertebrates to elevated seawater pCO₂. Published results are combined with new data and form a cross between a research and overview paper. The discussion of biological vs. physico-chemical control of calcification is a very important and exciting area of research in the ocean acidification community, and I encourage the authors to follow the general line of thought presented in this paper.

The main conclusion emphasized in the findings of this manuscript is that, contrary to popular prediction, calcification can increase in acidified water (Page 2268, Lines 8-9). However, the primary data index presented in Figs. 1A, B, D and Fig. 2 does not pro-

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vide information on calcification rate, but instead on the ratio of calcium to shell mass, so effectively, shell composition. Also, important methods and results components are missing in the manuscript. The data, as it is currently presented, does not support the conclusions made in the discussion. Before a more detailed review can take place, the following major revision points need to be addressed:

A) Calcification index:

The index $[Ca^{2+}] / (\% \text{ of total } CaCO_3 \text{ material})$, that is used as an indicator for calcification for several of the species, describes the calcium fraction of a given unit of shell mass and is not an indicator of calcification rate. For example, hypothetical *Patella vulgata* individuals that are characterized by an index of 50% (e.g. 2mg Ca²⁺ L⁻¹ / 4mg shell L⁻¹) at the beginning of an experiment. After several weeks of exposure to acidified conditions, both control and CO₂ exposed limpets can still have an index of 50% even though net calcification rate differed radically (e.g. control animal: 5mg Ca²⁺ L⁻¹ / 10mg shell L⁻¹, CO₂ exposed animal: 3mg Ca²⁺ L⁻¹ / 6mg shell L⁻¹). The clear distinction between data reporting shell composition, versus net calcification, is imperative for the discussion of this manuscript.

The conclusions that can be drawn from this index are restricted to the composition of the sampled structure which could have potentially changed during exposure to elevated pCO₂. For example, in Fig. 1B of this manuscript, the approx. 10% decrease in the index of pH 6.8 incubated shells reflects the decrease in CaCO₃ (due to dissolution) in relation to stable organic matrix levels. Changes in calcified structure composition, measured with a similar method, are nicely discussed in Spicer and Eriksson (2003, Fig. 5B).

Potential changes in the composition of calcified structures mineralized during acidification have not been closely examined to date, and inspire many interesting questions. It appears that there is a significant change in the composition of the shells of *Patella vulgata* (Fig. 1A of this manuscript), this finding should be discussed.

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B) Calcification rate

In order to draw conclusions about potential increases in calcification rate the net amount of mineralized CaCO₃ must be considered. It is unclear why the data for net CaCO₃ accretion (shell mass) and/or absolute Ca content of the entire shells are not presented for the various experimental groups in Figs. 1A, B, D and Fig. 2. While Fig. 1C does list some morphometric parameters for the snail *Littorina*, it does not give the change in shell mass / CaCO₃, which would be necessary to calculate calcification rates. This data needs to be added to allow a meaningful analysis. Fig. 1C should also be represented in such a manner that the large % differences between control and experimental groups discussed in the text (Page 2273, Line 15-19) are recognizable.

C) Methods The method used to measure calcium content is not adequately described in Section 2.2. The methods citation of Spicer and Eriksson 2003 describes sample preparation with nitric acid on page 224 (which differs from that of this manuscript) however, the actual measurement of the samples on an atomic absorption spectrophotometer is not described in Spicer and Eriksson 2003. This needs to be added. In addition, the accuracy / precision need to be discussed in order to judge what differences in shell CaCO₃ can be resolved with this method. What increase in shell mass is necessary to resolve a difference between treatments?

The following information is crucial to the reporting of long-term whole animal experimental trials and should be included in the manuscript for each species, and not just referenced from existing publications:

- duration of the exposure
- initial mass/size of the organism/structure
- feeding regime (e.g. algal densities / flow rates for filtrators, grazers?)
- season
- somatic / reproductive growth during the exposure period.

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How do the growth rates/calcification rates of the organisms in this manuscript compare to existing studies and field data? This information could be nicely compiled in a Table.

D) Statistics The statistics section of this manuscript needs to be expanded. Significant differences should be noted in the figures, and test results should be reported.

E) Manuscript structure The last paragraph of the Results, starting on Page 2273, Line 25, belongs in the Discussion. Moving this section of text could help restructure the Discussion in a way where it more closely follows the experimental results.

Interactive comment on Biogeosciences Discuss., 6, 2267, 2009.