

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

**HS Findlay**

hefi@pml.ac.uk

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Authors' response to comments

Findlay et al.

General reply to both referee #1 and #2

The main point raised by both referees concerns the appropriateness of measuring calcium within a calcified structure to draw conclusions on the calcification ability of that organism. Prior to addressing the points raised by the reviewers, we first wish to justify the use of this methodology.

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Both reviewers state that we did not measure calcification, instead the shell composition. Calcification is defined as 'a process that impregnates something with calcium (or calcium salts)' by Princeton University's Wordnet lexical database, and the Oxford Dictionary states that to calcify is to 'harden by deposition of calcium carbonate or other calcium salts'. The studies presented in this manuscript measure shell composition as an endpoint proxy of calcification. If calcification is the process by which calcium is added to an organic matrix, then a sample containing 40% calcium has been subject to more calcification than a sample containing 20% calcium, assuming they both started with the same (lower, e.g. 10%) calcium content.

Due to the destructive nature of the methodology there is no direct before and after comparison of calcium levels for the same individuals prior and post exposure to acidified conditions. Rather, as is frequently the case in experimental research, a randomly selected subset of individuals were placed under control conditions; thus the calcium levels obtained from this group represents typical proportions of calcium found in the calcified structures (shells or arms, species dependant) of the entire studied group. It can then be assumed that the sub-sample exposed to lowered pH conditions began the experimental exposure with calcium levels comparable to levels measured in the control group. Thus at the end of the experiment, when calcium levels (standardised as percentage per gram of shell/arm) are measured, any increase from the control is the product of the calcification process, for it is calcification that results in calcium deposition into the organic shell/skeletal matrix. As all the treatments had the same exposure time it is implicit that the calcification rates had to be different in each treatment to give the different values observed for final Ca content.

The methodology used in this study was used as a measure of calcification by Spicer & Eriksson (2003) to measure calcification in lobster larvae, and has also been used in several other published works as a measure of calcification. Further more if the objection is based on the premise that calcium is not representative of calcium carbonate, then this would also invalidate Ca45 labelling; another well used and published method

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for tracking calcification with protocols dating from the 1970's (e.g. Bohm, 1978, Sorrosa et al. 2005, Al Horani et al. 2007). The main difference between our methods and that of Ca45 is that the amount of calcium taken up during a given period can be distinguished in the latter, rather than using a control group to estimate previous levels of calcium content as we have done here. However, by using statistically adequate sample sizes, we are confident that our observed results are a function of the experimental conditions.

Because Referee #2 appeared to have a similar viewpoint to Referee #1 we have addressed the latter who provided somewhat more detailed comments, therefore please see the response to referee #1 for further comments.

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