Biogeosciences Discuss., 6, C391–C395, 2009
www.biogeosciences-discuss.net/6/C391/2009/
© Author(s) 2009. This work is distributed under
the Creative Commons Attribute 3.0 License.

Biogeosciences Discussions

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

Received and published: 12 May 2009

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.

Full Screen / Esc

Printer-friendly Version

Interactive Discussion

Discussion Paper

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

BGD

6, C391–C395, 2009

CC BY

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.

Below we have addressed the individual points raised by the referees. Because Referee #2 appeared to have a similar viewpoint to Referee #1 we have addressed the latter who provided somewhat more detailed comments.

Reply to Referee #1:

We firstly thank Referee #1 for their comments as we feel they have highlighted some areas where we have been misunderstand and that need clarification; and also has provided us with some very useful comments that will further improve our manuscript.

(A) Calcification index Calcification is the process in which mineral calcium builds up in soft tissue (as described above). Hence the use of calcium fraction in a given unit of shell provides an indication of the amount of calcification that has occurred. We do not measure calcification rate, as we were not able to follow the level of calcium through time from the start of the experiment to the end of the experiment in each treatment. As we attempt to explain in the introduction, the term net calcification used in this manuscript takes into account any biogenic laying-down of calcium carbonate and any dissolution of the shell; both of which will affect the mineral structure (and the amount of calcium carbonate) of the shell. The data in this manuscript are all relative end points of experiments and we therefore compare levels of calcium in shells exposed to different levels of CO2. Hence we present relative values and not absolute values, which is why we use the calcium concentration as a percent of total material, so that they are comparable.

BGD

6, C391–C395, 2009

CC BY

We realise now that in the discussion we often refer to rates but we would be happy to amend this to discuss calcification and not the rate at which it occurs. We realise this has caused confusion and may detract from the conclusions. We feel that with these amendments, the majority of the discussion is still valid and we have high confidence in our conclusions.

The referees comment: "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. . .." is correct and this is what we have attempted to address in the paper, however we would point out that the composition of the structure is the result of calcification (as explained in our general reply comment above). As mentioned above we did not measure rates and we compare treatment with treatment and "biology" with "no biology". We fully admit that using the term "rate" in the discussion was a mistake, as it appears to have caused much confusion (perhaps a naivety on our part that this was inferred and not measured). We feel this can be easily amended and does not change the main conclusions such as: animals are still able to calcify in acidified conditions, dissolution does occur in these experiments and differences are seen in the mineral composition (i.e. calcified structure) over relatively short periods of time. Spicer & Eriksson (2003) use the same methodology as in our manuscript and use the term "calcification".

We will add more discussion of the changes in composition of the shells of Patella, which the referee has emphasised and feel this would only enhance the discussion.

(B) Calcification rate We hope that in answering (A), as to why that index of calcification was used, we have also answered most of the comments in (B). Calcification rate was not measured. We are unable to show absolute Ca content in entire shells because we were not able to use the entire shell in the Ca analysis, as well as not being able to sample for calcium content over a number of time points including the start point.

With respect to the Littorina data (Fig 1c) we will alter the scale such that the differences

BGD

6, C391–C395, 2009

are recognizable.

(C) Methods Spicer & Eriksson (2003) describe sample dilution in nitric acid, which is the exact method we used in this manuscript (P 2272, L 16). Where our methodology differs from that of Spicer & Eriksson (2003) is that we state that we used atomic absorption spectrophotometer (AAS), whereas Spicer & Eriksson have not included this information in their methodology despite using this machine. The specific type of AAS machine that we used was precise to 1-2% RSD, with an accuracy of 10%. We will add this information into the revised manuscript.

The additional information requested by this referee (for both methods and also comparisons of "normal" rates) is, as is suggested, easily compiled into a table and will also be included in a revised manuscript. Although we emphasise again that we are not measuring calcification rate therefore we are hesitant to compare other study/field data of calcification rates, as this might cause more confusion.

(D) Statistics have been carried out on all the data in the manuscript and will be added to the methods and results as well as figures in a revised manuscript.

(E) We agree this paragraph would be better suited in the discussion and will rearrange this in a revised manuscript.

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