Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2018-332-RC2, 2018

Biogeosciences Discussions

Interactive comment on "Variation in brachiopod microstructure and isotope geochemistry under low pH–ocean acidification–conditions" *by* Facheng Ye et al.

Anonymous Referee #2

Received and published: 19 September 2018

Dear Editor,

The manuscript 'Variation in brachiopod microstructure and isotope geochemistry under low pH–ocean acidification–conditions' examines the change to micro-structure and biogeochemistry of the shell of the brachiopod Magellania venosa in natural conditions versus experimentally cultured brachiopods under low pH environments. The authors apply scanning electron microscopy (SEM) to understand changes to the microstructure in the form of the distribution of endopunctae in the anterior margin and the thickness of the primary layer. In addition, the study applies stable carbon and oxygen isotopes (δ18O, δ13C) to relate the shell growth to the surrounding seawater

chemistry in which the brachiopods were grown. This is a thorough investigation of the changes to the brachiopod shell formation under low pH environments. The authors comment from a biogeochemistry point of view relating the changing microstructure and isotope geochemistry of the shell to environmental carbon isotopes therefore providing further evidence for brachiopods to be useful as an ocean acidification proxy in geological samples. A very good multi-disciplinary approach to determine the impacts on the brachiopods shell growth.

I do have a few concerns in the way the samples were prepared using 5% acid etching, and if this could possibly mask the impacts of the experimental acidification on the microstructure. However, these are minor concerns outlined below for the authors. The use of calcein staining to mark the new growth of the shell distinguishes where natural and low pH environments impact the shell growth in the brachiopod. This should ensure that the authors can identify any similarities in the low pH treatment versus the acid etching. However, this should be discussed in the manuscript and perhaps guide the reader to the nice figures representing this. For example, can the authors provide figures 4 for each treatment for comparison? These are very nice visual representations of how the brachiopod microstructure is affected under low pH treatments versus the natural growth ahead of the calcein staining.

The manuscript is appropriate and well-suited for publication in Biogeosciences Discussions, I would recommend for publication with minor edits as detailed below.

Minor comments to the authors.

Introduction Please re-phrase, 'pH has dropped by 0.1 pH units and will probably drop another 0.3-0.5', these are projections based on modelling of historic data, I would suggest predicted or projected instead of probably. Line 15 states 'calcifying organisms', the table 1 refers only to a few brachiopod studies, please change to brachiopods. I could not comment on the supplementary table here. I would instead suggest including a sentence referencing some of the key papers outlining the consequences of exper-

imental acidification on biomineral formation in other calcifying organisms. There are such studies examining acidification impact on the microstructure of the sea urchin spicules for example Bray et al., 2014 (Med. Mar. Sci.), PUPA Gilberts group including studies by Politi et al., the authors only list here studies applied to molluscs.

Materials and methods. Page 8, line 2, how long were the brachiopods acclimated for prior to calcein staining and CO2 induced acidification? Low-pH culture of several brachiopods was done under two phases, what was the justification for this? Were these two phases comparable in their treatments? In general, the treatments appear clear in the table 2 and 3, however it is difficult to understand the experimental design without the details which are currently not obtainable from Jurikova et al., in review.

Microstructural analyses – this section is much easier to understand with sufficient detail for the reader to reproduce. The authors used 5% hydrochloric acid for etching the shell prior to SEM analyses. Although a standard protocol for SEM imaging, the authors should comment on how they can be sure that this has not affected the microstructure in comparison to the experimental acidification of the culture. How would the impact the microstructure be distinguishable compared to the acid etch of the microstructure of shells? Figure 4 and 5, can the authors please provide a specimen reference to which sample and treatment these images relate to? The manuscript suggests just two specimens #8005 and #9006 were used for these analyses. Can the authors conclude that these are representable as a sample population? Are these images available in supplementary information for comparison?

Carbonate stable isotopes analyses Likewise, the authors use 5% hydrochloric acid to clean shell prior to sample preparation for stable isotopes. Please detail why this was used, for example to remove organic material? If so why was a bleach or plasma ash treatment not chosen for this purpose to avoid potential issues with comparing experimental acidification treatments with hydrochloric acid treated shells? Section 3.3.2 During culturing Page 23, the authors state that 'The results from specimens (#43 and #63) grown under low pH conditions (pH3 and pH4) for a short time interval

of 214 days are difficult to interpret, as in this case, there is no direct control experiment sample to compare', can the authors confidently relate the changing microstructure and geochemistry here to acidification is the only comparison are those samples grown under natural conditions? It appears that the experimental treatments here are similar despite the pH used (Figure 9). I would question the relevance of this section, perhaps omit or justify how this is comparable.

Section 3.4 Stable isotopes Nice figure 10, it is clear to see trends between three specimens for significantly lighter stable carbon isotopes with experimental low-pH treatment compared to natural versus control treatments. Did the authors compare these data statistically?

Discussion Page 28, 'electron back scattering diffraction' should be electron backscatter diffraction. Line 20, 'May this indicate a greater amount of organic components in this part of the shell?' is this what the authors suggest? Please rephrase not as a question but a statement with references or omit. Lines 28, 'living organism' this should be living organisms. Page 34-35. The discussion of the depleted δ18O, δ13C is related to changes in percentage, can the authors present the statistical significance here of the changing isotope values? The authors state that there is individual specimen variability, does this remove the significance of the low pH treatment over the isotope depleted values? Or are the authors suggesting here that there are insufficient specimen numbers to make significant statements relating to the isotope data? Page 35, line 11 'More measurements are however needed to fully answer this.'? Page 35, line 16 'Thus, we think', perhaps the data suggest? The authors end in the statement 'secondary layer isotope record may reflect the environmental conditions supporting the interpretation of brachiopod shells as good archives of geochemical proxies, even when stressed by ocean acidification.'. This is also stated in the abstract as one of the main implications of this study. Following the current discussion on page 34-35 I would question whether the authors can make this statement, and whether there is sufficient evidence to support this, although Figure 10 does suggest this is the case. Please directly refer to the

data here; are there sufficient samples, what is the n-number? This will enable the reader to determine if the manuscripts data do support these conclusions. If this data is not available then the authors will need to remove this emphasis from the abstract and conclusion statements. Page 35 conclusions 'This was related to the source of carbon dioxide gas used in the culture setup', could this not be due to a change in the carbonate compositions as a result of adding CO2 impacting the DIC? Did you test the carbon isotopes of the gas? I have seen this drop in carbon isotopes in the natural seawater samples where increasing CO2 from run-off caused a lighter carbon isotope value. The authors should expand this discussion to the previous paragraph.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2018-332, 2018.