

## ***Interactive comment on “ $\delta^{11}\text{B}$ as monitor of calcification site pH in marine calcifying organisms” by Jill N. Sutton et al.***

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### General Comments

Sutton *et al.* present boron isotopic composition measurements of marine calcifying organisms cultured under controlled conditions using modifications to existing MC-ICP-MS measurement procedures. These are interesting results worthy of publication after revision. In addition to the detailed comments below, I have three general recommendations.

1) Discussion of sample collection, subsampling, and what the different isotope measurements were measured on. Namely, there isn't any information, so it is impossible to tell whether multiple specimens were used, whether each specimen was subsam-

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pled in the same skeletal region, etc. This must be included in an expanded Materials/Methods section.

2) Wording of boron isotope differences in the study. This study devotes much attention to the fractionation between boric acid and borate in aqueous solution, termed the fractionation factor ( $\alpha_B$ ). However, the study also confusingly defines their differences between the  $\delta^{11}\text{B}$  of carbonates and their expected  $\delta^{11}\text{B}$  based on the  $\delta^{11}\text{B}$  of borate ion in solution as “fractionations”.

This is an unnecessary complication. The data of this study do not address the actual fractionation factor ( $\alpha_B$ ), which has been determined, but rather address how carbonate  $\delta^{11}\text{B}$  values may be offset from the  $\delta^{11}\text{B}$  of borate ion in solution. Put another way, this study tests the assumed model that carbonate  $\delta^{11}\text{B}$  records seawater pH via sole incorporation of borate ion from seawater at the measured seawater pH. Carbonate data that are discordant with this model (as in this study) do not necessarily imply any isotopic fractionations; instead, they suggest that one of the assumptions of the model may be wrong when applied to the carbonate in question.

3) The discussion of factors influencing the quantification of calcifying fluid pH needs to be refocused/expanded. The major strength of this study is that all calcifiers were grown under approximately the same conditions. This experimental design effectively minimizes uncertainty arising from variations in  $\text{pK}_B^*$  and  $\alpha_B$ . However, the only sources of uncertainty to  $\text{pH}_{cs}$  discussed are  $\text{pK}_B^*$  and  $\alpha_B$ , exactly those that are best controlled. This discussion needs to be expanded to evaluate the effects of known modifications to each carbonate’s microenvironment and the possibility of alternate boron incorporation pathways other than borate (particularly for the coralline alga).

Specific comments/Technical corrections:

L15: Suggest change opening sentence to “The boron isotopic composition ( $\delta^{11}\text{B}$ ) of marine biogenic carbonates. . .” and remove  $\delta^{11}\text{B}$  reference on L18

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L33/34: Cite original studies of instrumental pH records in lieu/addition to IPCC; e.g., BATS (Bates, 2007); ESOTC (Gonzalez-Davila et al., 2010), and ALOHA (Dore et al., 2009) or more recent studies

L45-50: The theoretical model for boron incorporation predates Pagani et al. (2005). Please cite original studies; e.g. Hemming and Hanson (1992) for  $\text{CaCO}_3$   $\delta^{11}\text{B}$  reflecting  $\delta^{11}\text{B}$  borate, and Zeebe and Wolf-Gladrow (2001) for description of parameters needed to calculate pH from  $\delta^{11}\text{B}$ .

L46-47: The definition of  $\delta^{11}\text{B}_{sw}$  is misleading;  $\delta^{11}\text{B}_{sw}$  is the isotopic composition of boron in seawater (e.g., L49), which reflects the sum of all boron species in seawater. However, on L46 the text states the boron isotopic composition of borate in seawater. This is not  $\delta^{11}\text{B}_{sw}$ , but instead is defined separately ( $\delta^{11}\text{B}_{borate}$  or similar) and is a function of both  $\delta^{11}\text{B}_{sw}$  and pH.

L58: Catanzaro et al. (1970): Boric Acid: Isotopic and Assay Standard Reference Materials is the appropriate reference for NIST 951.

L59: “constant” instead of “consistent”

L64: “was” instead of “has been”. Since the identification of errors in Kakihana’s vibrational spectra (L69), the Kakihana fractionation is not appropriate and is not used for  $\delta^{11}\text{B}$ -pH applications. Hönisch et al. (2007) (response to Pagani et al., 2005) discusses the fractionation and the concept of species-specific calibrations in more detail.

L72-76: Reword this. The studies referenced on L75-76 do not argue for different fractionation factors; rather, they argue for species-specific calibrations between  $\delta^{11}\text{B}_{\text{CaCO}_3}$  and seawater pH. I am unaware of any evidence that the isotopic fractionation between boric acid and borate (the fractionation factor) is fundamentally different in biogenic calcifying fluids than in seawater. Moreover, any insights of calcifying fluid pH require assuming that the same fractionation factor applies in both seawater and calcifying fluid (e.g., Trotter et al., 2011).

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Section 1.1 could use greater clarity for pH terms. The manuscript starts with discussing seawater pH, and  $\delta^{11}\text{B}$  as a seawater pH proxy, but transitions to calcifying fluid pH in this section. For the sake of readability, I suggest you define these separately here (seawater pH =  $\text{pH}_{sw}$  and calcifying fluid pH =  $\text{pH}_{cf}$ , or similar), and use throughout the text.

L83-85: This is true, although you could additionally cite several recent reinterpretations of boron incorporation into carbonates (Norieaux et al., 2015; Uchikawa et al., 2015, Balan et al., 2016).

L94-95: Suggest rewording to “organisms’ ability to regulate pH at their site of calcification” L108:  $\text{Ca}^{2+}$

L109-125: This would be better placed at the beginning of Section 1.2, before the discussion of OA reducing  $[\text{CO}_3^{2-}]$

L125: Specify that  $K^*_{sp}$  is a function of temperature

L136: Is there a section 1.3? If not, change this to “1.3”

L137-138: Rephrase to “may record” and cite studies suggesting that carbonate  $\delta^{11}\text{B}$  records calcifying fluid pH. While it has been hypothesized that  $\delta^{11}\text{B}$  records pH in the calcifying microenvironment, to say that  $\delta^{11}\text{B}_{CaCO_3}$  should record calcifying microenvironment pH is a stretch given current uncertainties in how pH is controlled in these microenvironments (e.g., Section 1.2), and uncertainties in the  $\delta^{11}\text{B}$  proxy (see discussion in Farmer et al., 2015).

L164-171: Move to Methods section as a “Materials” subsection. Also, how were the specimens subsampled for isotopic analysis? Were they bulk homogenized or subsampled on particular growth features? Is there only one specimen per taxa or multiple specimens? This is very important to include as it might shed light on some of the poor reproducibility you observe (especially for the coralline alga).

L198-200: You can remove the sentence starting with “All samples. . .” since you dis-

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cuss this immediately below.

L209: Were the samples just rinsed in buffered UHQ water, or were they stored in the water? It is unclear if they were then acidified in this water medium (or not).

L224 (Batch method): Can you specify the type of microcentrifuge tubes used (polypropylene vs. PFA/Teflon), and whether the tubes were reused between samples? Or did you transfer the resin into separate microcentrifuge tubes for each sample? If the latter, how did you store the resin between samples?

L252: “The  $\delta^{11}\text{B}$  was also evaluated. . .” Please explain. This reads as if you used the internal carbonate standards to correct your  $\delta^{11}\text{B}$  values, which would not be appropriate. As these are internal standards, do you mean to say that you are using them to evaluate the efficacy of the preparation and measurement protocol?

L285-286: Unnecessary subsection; please remove.

L308-322: Based on Table 3, it seems that batch separation with  $\text{NH}_3$  injection was most commonly used. Does that reflect your experience with the different methods and which one seemed most replicable and user friendly? Can you make a recommendation on which separation and injection methodology you think other others should follow?

L320: Can you comment on how much lower the batch method blanks were? The procedural blanks are listed as sub-nanogram (L235), but I cannot find a distinction between column vs. batch protocols.

L334-360: Multiple references to a phantom Figure 5. Please check Figure references throughout text.

L360 (Coralline red alga): Please comment on why the  $\delta^{11}\text{B}$  values for these specimens are so different ( $\pm 3.7$  per mil uncertainty is massive!).

L368-372. Couldn't both be possible-e.g., microenvironment pH adjustment and boric

acid incorporation? If that was the case, could you actually determine the pH at the site of calcification? I'd strongly recommend including a figure and calculations showing how the derived value of pH<sub>cs</sub> would change as a function of varying % boric acid incorporation.

Moreover, pH<sub>cs</sub>=9.4 seems pretty extreme. Is there any evidence for a physiological advantage to a calcifying organism obtaining such alkaline pH in its calcifying medium? I don't disagree with the proposed mechanism (algal photosynthesis), just the magnitude. I imagine that at this pH, CaCO<sub>3</sub> would spontaneously precipitate (due to massively high omega), which would not be desirable for the organism.

Finally, note that NMR is not useful for quantifying % boric acid incorporation (see and reference Balan et al., "First-principles study of boron speciation in calcite and aragonite" GCA 193, 2016).

Section 4.3: Seems out of order. I find it more intuitive to present the equation for pH calculation first (L455-460), then discuss/test the assumptions of this approach (Section 4.3.2), then finally loop around to the best estimate of pH<sub>cs</sub> and comparison to OA responses.

L426: This is not the correct terminology. This study's data do not suggest that the B isotope fractionation is species dependent; there is no direct measurement of the fractionation in this study. Rather, these data suggest that the B isotope composition of these taxa cannot be explained solely by borate incorporation at ambient seawater pH.

L430: Specify the assumption that only borate is incorporated here

L449: "by testing the factors that may influence the theoretical model of borate  $\delta^{11}\text{B}$  variation as a function of pH"

The theoretical model of carbonate  $\delta^{11}\text{B}$  reflecting seawater pH has three parts: 1) borate  $\delta^{11}\text{B}$  varies with pH in a known fashion (requires knowledge of pH,  $\text{p}K_B^*$  as you say

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here); 2) carbonates are calcified from unmodified seawater; 3) boron in carbonates results from solely borate incorporation. Only by combining all three assumptions can you use carbonate  $\delta^{11}\text{B}$  to record seawater pH. You've discussed 1; please discuss what your data suggest about 2 and 3, and how uncertainties in these assumptions could influence your data. You've already discussed 2 (modification of calcification site chemistry) throughout the discussion; bring it all together here.

L475-495: The first paragraph (L475-486) is just rehashing the introduction and can be removed. The second paragraph (L487-495) is the meat of this.

L478: Figure 2 instead of Figure 3?

L499: I would urge caution with this relationship between pH elevation and OA response, as it is at best a qualitative relationship. Also, seeing as this is a central point of the manuscript, I would recommend including a figure to illustrate the relationship between pH elevation and OA response (something like Doney et al. 2009's Figure 4 may work). L505: Please note that this "species-specific" calibration approach is not new; it has been the standard procedure in the boron isotope community for years, as demonstrated by numerous studies that should be cited here (e.g., Sanyal et al., 1996; 2001; Hönisch et al., 2003; Trotter et al., 2011; Anagnostou et al., 2012; etc.)

Figure 2: -The seawater borate curves must be mislabeled; all else being equal, increasing alpha will lead to a lower  $\delta^{11}\text{B}$ -borate at lower pH. I think the dotted line should be Kakihana and the solid line should be Klochko (see also Fig. 4).

-Why did you choose to plot these specific data? The chosen ones seem quite random, and there are many other data out there worth considering (as your Table 5 illustrates) that may be most appropriate for comparison with the carbonates presented in this study.

-Is the large pH range on the x-axis (7-10) necessary? It is difficult to make out the individual studies.

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-Note also typos: “Hönisch”, “Brachiopod” and “Penman”.

Figures 2 and 4: Please also plot  $\delta^{11}\text{B}$  – boric acid for the fractionations.

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