

## ***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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We wish to thank Dr. Jesse Farmer for their thorough review of our manuscript and their helpful comments. We believe that we can address all of the major comments indicated by Dr. Farmer as indicated in the discussion below.

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

Author response: We will include this information.

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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  $\delta_{11B}$  of carbonates and their expected  $\delta_{11B}$  based on the  $\delta_{11B}$  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  $\delta_{11B}$  values may be offset from the  $\delta_{11B}$  of borate ion in solution. Put another way, this study tests the assumed model that carbonate  $\delta_{11B}$  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.

Author response: We can address this comment as discussed below in the responses to more specific comments.

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  $pK_B^*$  and  $\alpha_B$ . However, the only sources of uncertainty to pH<sub>c</sub>s discussed are  $pK_B^*$  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).

Author response: We can address this complication as discussed below in the responses to more specific comments.

More specific comments L15: Suggest change opening sentence to “The boron isotopic composition ( $\delta_{11B}$ ) of marine biogenic carbonates: : :” and remove  $\delta_{11B}$  refer-

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ence on L18 L33/34: Cite original studies of instrumental pH records in lieu/addition to IPCC; e.g., BATS (Bates, 2007); ESOTC (Gonzalez-Davlia 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  $\delta^{11}\text{B}$ .

Author response to above comments: We will change this information as recommended by the reviewer. We will cite Byrne et al. (2010); Vázquez-Rodríguez et al. (2012) in L33, Feely et al. (2016); Feely et al. (2008) in L34

L46-47: The definition of  $\delta^{11}\text{B}_{\text{sw}}$  is misleading;  $\delta^{11}\text{B}_{\text{sw}}$  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  $\delta^{11}\text{B}_{\text{sw}}$ , but instead is defined separately ( $\delta^{11}\text{B}_{\text{borate}}$  or similar) and is a function of both  $\delta^{11}\text{B}_{\text{sw}}$  and pH.

Author response to above comments: This should read “ $\delta^{11}\text{B}$  composition of borate in seawater ( $\delta^{11}\text{B}(\text{OH})_4$ ; Pagani et al., 2005)” and will be changed.

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 Kakhana’s vibrational spectra (L69), the Kakhana 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.

Author response to above comments (L58-L64): We will make these changes as recommended by the reviewer.

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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  $\delta^{11}\text{BCaCO}_3$  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).

Author response to above comments: We will change this information to “Moreover, due to the ability of some calcifying organisms to alter carbonate chemistry at their site of calcification, empirical species-specific calibrations between  $\delta^{11}\text{BCaCO}_3$  and seawater pH are likely more appropriate than theoretical  $\alpha$  values if the goal is to reconstruct ambient seawater conditions (Anagnostou et al., 2012; Hönlisch et al., 2004; Krief et al., 2010; Rae et al., 2011; Reynaud et al., 2004; Trotter et al., 2011).

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 =  $\text{pH}_{\text{sw}}$  and calcifying fluid pH =  $\text{pH}_{\text{cf}}$ , 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  $[\text{CO}_2]$

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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  $\delta_{11B}$  records calcifying fluid pH. While it has been hypothesized that  $\delta_{11B}$  records pH in the calcifying microenvironment, to say that  $\delta_{11B}CaCO_3$  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  $\delta_{11B}$  proxy (see discussion in Farmer et al., 2015).

Author response to above comments (section 1.1. to L138): We will change this information as recommended by the reviewer. For line 125 we will specify that  $K_{sp}$  is a function of temperature and salinity in the text. For L137-L138, we will rephrase the sentence with “may record” and we will cite the following papers (McCulloch et al. 2012; Holcomb et al. 2014; Farmer et al. 2015; Martin et al. 2016)

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).

Author response: We will add a new materials section and provide a short description of how the samples were sampled. Part of the information is included in Ries et al. (2009), but we will include a short description to clarify. The samples were subsampled on new growth, homogenized, and multiple specimens per taxa were evaluated (as indicated by the name of the sample in table 3). The extent of new growth was evaluated based on the addition of a barium spike as described in Ries (2011) and this information was used to guide subsampling. We will also clarify the differences between the intra-specific (same species but different organisms), intra-organism (sub-sampling the same organism), and analytical reproducibility. As the reviewer noted, the intra-specific

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reproducibility for the red coralline alga is large, however, the intra-organism and analytical reproducibility is not. This suggests that there is likely geochemical heterogeneity in the carbonate matrices of this species, with large variability observed between organisms but the analytical reproducibility is robust. However this important point will be highlighted and better explained in the text, as it was also noted by Reviewer 3.

L198-200: You can remove the sentence starting with “All samples: : :” since you discuss this immediately below.

Author response to above comments: We will change this information as recommended by the reviewer.

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).

Author response to above comment: The samples were simply rinsed in the water. We will change this sentence to: “Samples were then ultrasonicated for 10 minutes, centrifuged, and then the acid was removed. The samples were washed twice with pH- buffered UHQ water, centrifuged and the water was removed.

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?

Author response to above comment: We used polypropylene tubes and they were not used between samples. The resin was prepared following the description on lines 194-195: “The resin was crushed and sieved to a desired 100 – 200 mesh, then cleaned and conditioned to a pH of 7 (6.8 – 7.2).” We then take a small aliquot of the resin and place it into the polypropylene microcentrifuge tubes, which are then washed and prepared further. See lines 225-227 “Cleaned samples (pH 7) were transferred into acid-cleaned micro-centrifuge tubes (500 uL) containing 5 mg of resin, which is B-cleaned with 500

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$\mu\text{L}$  of 0.5 M  $\text{HNO}_3$ , and then rinsed with 500  $\mu\text{L}$  of MQ water (buffered to pH 7 with 2%  $\text{NH}_4\text{OH}$ ) three times to elute the other cations in the matrix and achieve pH 7.”

For clarity, we will make the appropriate links between these two sections and indicate that they are indeed polypropylene tubes.

L252: “The \_11B was also evaluated: : :” Please explain. This reads as if you used the internal carbonate standards to correct your \_11B 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?

Author response to above comments: These are not internal standards and were not used to correct our values. They are external reference standards that were analysed multiple times and were used to evaluate the method. The sentence will be changed to: “The  $\delta^{11}\text{B}$  of the external calcium carbonate standards JCp-1 (Porites sp.), NEP (Porites sp.) and Jct-1 (hard clam) were also evaluated, which were processed in the same manner and are reported in the results section (see Section 3.1.1) alongside their published reference values (Foster et al., 2013; McCulloch et al., 2014).”

L285-286: Unnecessary subsection; please remove.

Author response to above comments: Ok, this will be removed.

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?

Author response to above comments: We do discuss this point on lines 312-317.

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.

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Author response to above comments: We will add a comment here, but typically the column method had a blank that was near 0.5 ng and the batch method had blanks as low as 90 pg.

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

Author response to above comments: We will correct this.

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!).

Author response to above comments: We have already responded to this comment above, see lines 164-171. In short, the standard deviation represents individual-to-individual variation (i.e. vital effects) and not the analytical precision, which is robust.

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 pHcs would change as a function of varying % boric acid incorporation. Moreover, pHcs=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,  $\text{CaCO}_3$  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).

Author response to above comments: Yes, it could be a combination of two. If partial boric acid incorporation is the only source resulting in a high boron isotopic composition in coralline red algae, the portion of boric acid would need to be extremely high

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(>75 %), which contradicts the observations of inorganic or organic calcite (e.g. Cusack et al., 2015 estimated 30% trigonal B in the calcite lattice of a different species of coralline algae), therefore, we can not rule out the potential of pH up-regulation in this species. If 30% of the boric acid incorporated into the calcite, as suggested by Cusack et al. (2015), the calcification site pH will be still as high as 9. In addition, Short et al. (2015) observed that epiphytic turf algae can modify seawater chemistry (up to a pH of 9) within the diffusive boundary layer above coralline algal crusts, therefore a calculated pH of 9 is possible. We will expand our discussion on this section to make our arguments more clear on the points above and include a new figure (Figure 5; see attached) with the figure caption: “The influence of pH on the speciation of boron in seawater and  $\delta^{11}\text{B}$  (adapted from Rollion-Bard, 2011b). The solid and dashed curves represent the  $\delta^{11}\text{B}$  composition that would result from the incorporation of different amounts of  $\text{B}(\text{OH})_3$  into the marine carbonates. The dashed vertical lines represent the calculated pH based on the assumption that 0%  $\text{B}(\text{OH})_3$  is incorporated into temperature coral and 0%, 30% and 75%  $\text{B}(\text{OH})_3$  is incorporated into coralline alga.”

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 pHcs and comparison to OA responses.

Author response to above comments: We agree, the order can be modified to be clearer.

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.

Author response to above comments: We originally used this terminology since the B

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isotope composition of the seawater should be consistent throughout all the conditions, but the reviewer is correct in that this was not directly measured. We can use the recommendation of the reviewer to avoid ambiguity.

L430: Specify the assumption that only borate is incorporated here

Author response to above comments: We will correct this.

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,  $\text{pK}_b^*$  as you say 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.

Author response to above comments: We can modify this. We will ensure point 3 is addressed by referring to the expanded discussion on B incorporation (see response to lines 368-372).

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?

Author response to above comments: We will make these changes

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

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work).

Author response to above comments: We agree that a simple determination of calcifying fluid pH could not be considered a strong determinate of OA response, as ideally one would want to assess how CF responds when challenged with changing conditions. However data on OA sensitivity exists from previous work on our samples (e.g. Ries et al., 2009), therefore it made sense to include a reference and a brief discussion of that information here and in Table 4. We do not think it is necessary to include an illustration between pH elevation and OA response since this type of relationship was already analysed for our samples in Ries et al., 2009.

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.)

Author response to above comments: We will cite those papers, it was an oversight on our part to not include that information.

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. -Note also typos: “Hönisch”, “Brachiopod” and “Penman”. Figures 2 and 4: Please also plot  $\delta^{11}\text{B}$  – boric acid for the fractionations

Author response to above comments: We aim to show boron isotopic composition from some of the most studied marine biogenic carbonate archives including corals, foraminifera and bivalves. We also want to show that the data has been reported to

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follow different borate fractionation curves. Therefore, we have chosen studies that have more than two boron data points in a wide range of pH conditions, which aim to calibrate/validate the  $^{11}\text{B}$ -pH proxy in different species. For the above purpose, we will also replace the reference from Foster et al., 2008 to Sanyal et al., 1996 and Henehan et al., 2013. To incorporate all the data, the x-axis will range from 7- 9.5. It currently shows the limits of sensitivity of the borate curve to pH, which is relevant given where the coralline algae data fall.

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Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2017-154>, 2017.

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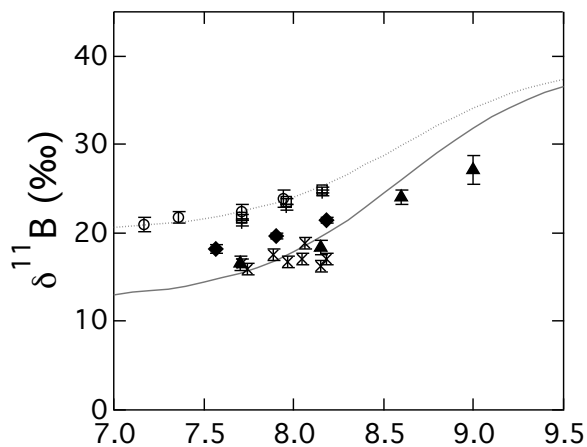
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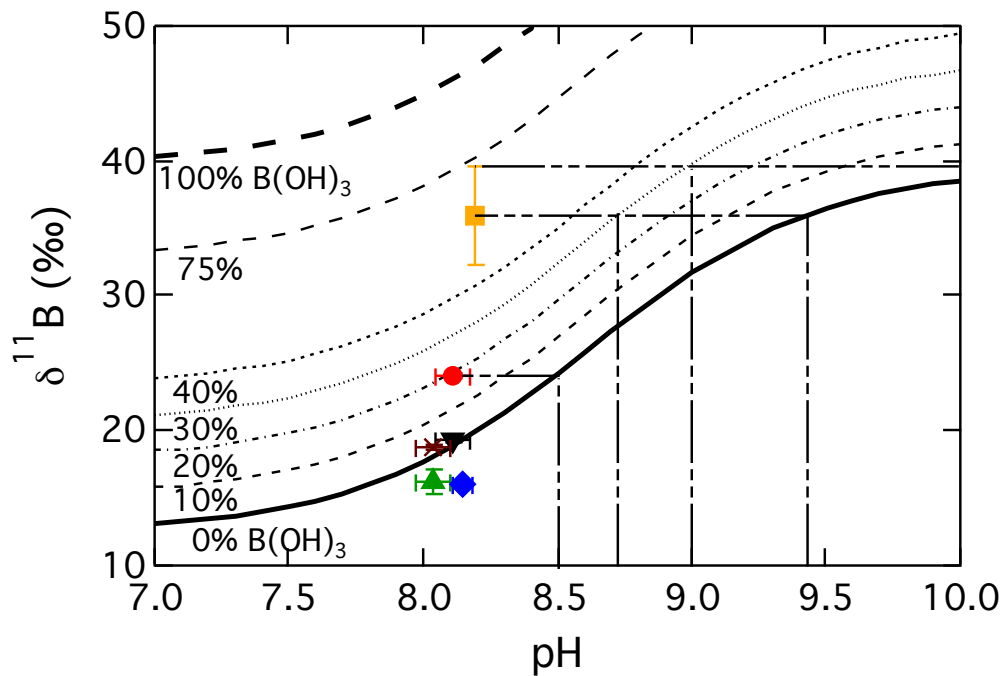
- ..... Seawater borate  $\text{pH}_{\text{sw}}$   
 $\alpha = 1.0194$  (Kakihana *et al.*, 1977)
- Seawater borate  
 $\alpha = 1.0272$  (Klochko *et al.*, 2006)
- + *Acropora nobilis* (Hönisch *et al.*, 2004)
- *Porites cylindrica* (Hönisch *et al.*, 2004)
- *Stylophora pistillata* (Holcomb *et al.*, 2014)
- ▲ *Orbulina universa* (Sanyal *et al.*, 1996)
- ◆ *Globigerinoides ruber* (Henehan *et al.*, 2013)
- × Brachiopod (Penman *et al.*, 2013)

Fig. 1. New Figure 2

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- Temperate coral
- Coralline red alga
- ▲ Temperate urchin
- ▼ Serpulid
- × Tropical urchin
- ◆ American oyster

Fig. 2. New Figure 5

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