Reply to reviewer 1

We wish to thank the reviewer for their succinct and thoughtful analysis of our manuscript and criticisms concerning the calculation of pH for calcite δ11B.

Our intention with this manuscript was to consider calcification site pH as the primary driver of the observed range in δ11B values for the evaluated species as a primary hypothesis, and to discuss whether the values we obtained made sense given what else is known about their biocalcification strategies including independent measurements of internal pH from other methods (eg. pH sensitive dyes, microelectrodes). Our intention was to set up this hypothesis as a straw man to see whether our data were constant with the idea of primary pH control across these diverse species, and if it was not then to discuss other factors that could be at play. At a number of points in the manuscript we also explore possible alternative explanations including mineralogical effects, boric acid incorporation, and we also discuss uncertainties in the calculation of an absolute pH value from carbonate δ11B values.

We agree with the reviewer that it is possible that there are additional complications to the interpretations in calcitic organisms. In particular, the possible incorporation of trigonal boron/boric acid into the skeleton and many of the studies mentioned are already referenced in the text. This was addressed to a degree in the original text. For example, lines 329-331 we state “Boron co-precipitation with inorganic CaCO3 (i.e. abiogenic) is known to be dependent on solution pH and inorganic CaCO3 precipitation rate, however, the relative abundances of the inorganic B species in solution that are incorporated into inorganic CaCO3 (borate ion and boric acid) have been shown to be independent of the parent solution pH (Mavromatis et al. 2015).” and also lines 365-370 where we state “…raising the possibility that coralline red alga incorporate both species of dissolved inorganic boron during calcification. In support of this argument, Cusack et al. (2015) provide NMR data indicating that 30 % of the B incorporated into the coralline red alga Lithothamnion glaciale was present as boric acid. However, since the coralline red algae were reared at a pH of 8.1, the δ11BCaCO3 compositions observed for the coralline alga in the present study would require incorporation of both inorganic species of boron at [B(OH)3]:[B(OH)4-] ratios of ca. 75:25, not the 30:70 ratio observed by Cusack et al. (2015).”

Nevertheless we agree that this information could be more prominently addressed in the main text and propose that a series of changes could be included to address this alternative hypothesis that particularly affects the calcite mineralizing organisms. For example, we can add a clarifying sentence in the abstract and provide more discussion to section 4.2.

We believe an interesting aspect of our study is that of the three calcitic organisms analyzed, only one of them has δ11B values (the coralline alga, 35.89 ± 3.71 ‰; n = 3) potentially consistent with boric acid incorporation, as suggested in Noireaux et al., 2015, EPSL, Balan et al., 2016, GCA; Branson et al., 2015, EPSL; Mavromatis et al., 2016, GCA. This possibility has also been explored in a NMR based study of coralline algae by Cusack et al. 2015 and we did cite this study on lines 366-370 and provide some discussion, as mentioned above. Nevertheless we agree with the reviewer that aspects of the text could be tightened up in this respect (for example clarifying the findings of Noireaux et al., 2015 as the reviewer highlighted).

The high-Mg calcite urchin species we studied have low δ11B values, with one species even having a value that is actually lower than the seawater borate δ11B value (the temperate
urchin *A. punctulata*; 16.28 ± 0.86 ‰; n = 3; Tables 3 and 4). Given these values in the two species of urchin-examined incorporation of significant amounts of seawater boric acid with much higher δ¹¹B values, seems unlikely.

In our manuscript we do have a separate section discussing biomineralization strategies of each organism studied (sections 4.2.1 to section 4.2.5), however in response to the reviewer’s comment we can certainly expand this.

We also accept that there are of course many previous studies reporting a range of δ¹¹B values in marine organisms. However we believe a striking finding of our study is the extreme range (20 per mil) we observed in species that were cultured in environmentally controlled and equivalent conditions.

*Lines 42-43, some of the references cited here are not related to boron isotopes, e.g. Saenger et al., 2013; Zinke et al., 2014.*

**Author Response:**
Originally these references were meant to indicate that corals have disequilibrium “vital” effects in other isotope systems but they can be removed from this sentence since it does not make sense to include them now.

*Lines 46-47 “¹¹B composition of borate in seawater (¹¹BSW; Pagani et al.,2005) ” ¹¹Bsw is commonly used to indicate boron isotope composition of seawater other than that of borate. Please modify.*

**Author Response:**
This should read “δ¹¹B composition of borate in seawater (δ¹¹B(OH)₄; Pagani et al.,2005)” and will be changed.

*Line 59, “The ¹¹B of modern seawater is 39.61 ± 0.04 ‰ (Foster et al., 2010)”*, 2SD should be used when reporting a replicated and certified value, so the value should be 39.61± 0.20 ‰ (Foster et al., 2010).

**Author Response:**
We will fix this.

*Lines 164-168: The setup of culturing experiment should be illustrated in details in the Methods and Materials Section, as well as the method to identify and separate the new growth part of each skeleton or shell for isotope measurement.*

**Author Response:**
We can certainly add more information on the culturing setup and information on the method used for new growth identification. To clarify, the cultures presented here were previously reported in another publication, Ries et al., 2009, so we feel it is appropriate to summarize important elements of the culturing setup rather than repeat the very detailed description in that paper.
Lines 168-171 This is a replicate of Section 2.3, and not an important part for the Introduction, so please remove.

Author Response: 
We can remove this.

Line 279 Is the analytical precision shown here 2SD or SD?

Author Response: 
The analytical precision shown here is 2SD, this will be mentioned in the text.

Lines 285-286 Please remove this section.

Author Response: 
We can remove this.

Lines 333-334 “polymorph mineralogy was not found to influence boron isotope fractionation (Noireaux et al. 2015).” This seems to be in contrary to the conclusion of Noireaux et al. 2015 who claim that “Our results indicate that the main controlling factors of 11B are the solution pH and the mineralogy of the precipitated carbonate mineral”.

Author Response: 
This is a glaring error on our part. We tried to simplify an argument, and the message was lost in translation. Thank you for picking up on this.

The sentence should read “Although Mavromatis et al. (2015) also found that polymorph mineralogy influences both the B/Ca ratio (higher in aragonite than calcite) and speciation of B in inorganic CaCO3 (borate/boric acid ratio higher in aragonite than calcite), B incorporation alone does not appear to influence boron isotope fractionation.

Lines 336-337 “if shell mineralogy was the primary driver of the observed interspecific variation in δ11BCaCO3 compositions – a trend that is not observed”. As suggested by Noireaux et al. 2015, both solution pH and mineralogy are important factors controlling 11B in carbonates, differences in calcifying fluid pH may also obscure “this mineralogical trend”, especially the underlying calcification mechanisms of each calcifier remain largely unknown. So, I don’t think mineralogical influences can be easily excluded.

Author Response: 
We have somewhat touched on this in the points above. We agree that the possibility of boric acid incorporation needs to be discussed. The coralline alga has a δ11B value potentially consistent with the mineralogical effect discussed, but not the urchin species.

Lines 368-370 With such high proportion of trigonal B incorporation, the classic 11BpH equation cannot be used to calculate the pH, as 11Bcarb = 11Bborate is the basic assumption for the calculation.
Author Response:
As we believe data from only one of our species is potentially (but not necessarily) consistent with significant trigonal B incorporation relative to borate (see responses above) we prefer to discuss this as uncertainty when calculating calcification site pH, adopting the straw man approach we described above.

*Line 426 but also mineralogy dependent*

Author Response:
We can add a qualifying addition here.

*Lines 427-432 The premise of using the equation mentioned in the paper to calculate pH by carbonate 11B is that borate ion is the only species that enters into the lattice (for example in aragonite). As suggested by both theoretical calculation and NMR experiment, both boric acid and borate ion exist in the lattice of calcite. Therefore, for those calcite organisms, this 11B-pH equation may not be applicable, and the calculated pH value may not reliably reflect the pH of calcifying fluid.*

Author response:
This point is largely covered in our response to previous comments.
Reply to reviewer 2

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.

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 (_B). However, the study also confusingly defines their differences between the _11B of carbonates and their expected _11B based on the _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 (_B), which has been determined, but rather address how carbonate _11B values may be offset from the _11B of borate ion in solution. Put another way, this study tests the assumed model that carbonate _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 pKB* and _B. However, the only sources of uncertainty to pHcs discussed are pKB* and _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 (_11B) of marine biogenic carbonates: : :” and remove _11B reference on L18

L33/34: Cite original studies of instrumental pH records in lieu/addition to IPCC; e.g., BATS (Bates, 2007); ESOTC (Gonzalez-Davilia 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 CaCO3 _11B reflecting _11B borate, and Zeebe and Wolf-Gladrow (2001) for description of parameters needed to calculate pH from _11B.

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

L46-47: The definition of _11Bsw is misleading; _11Bsw 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 _11Bsw, but instead is defined separately (_11Bborate or similar) and is a function of both _11Bsw and pH.

Author response to above comments:
This should read “_11B composition of borate in seawater (δ¹¹B(OH)₄; Pagani et al.,2005)” and will be changed.


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 _11B-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 change “was” instead of “has been” as recommended by the reviewer.

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 _11BCaCO3 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 δ\(^{11}\)B\(_{\text{CaCO}_3}\) and seawater pH are likely more appropriate than theoretical \(\alpha\) values if the goal is to reconstruct ambient seawater conditions (Anagnostou et al., 2012; Hönisch 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 \(_{\text{11}}\)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 = pH\(_{\text{sw}}\) and calcifying fluid pH = 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: Ca2+

L109-125: This would be better placed at the beginning of Section 1.2, before the discussion of OA reducing [CO2\(_{\text{aq}}\)]

L125: Specify that K\(_{\text{sp}}\) 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 \(_{\text{11}}\)B records calcifying fluid pH. While it has been hypothesized that \(_{\text{11}}\)B records pH in the calcifying microenvironment, to say that \(_{\text{11}}\)BCaCO\(_3\) 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 \(_{\text{11}}\)B 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\(_{\text{sp}}\) 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
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 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 dis- cuss 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 μL) containing 5 mg of resin, which is B-cleaned with 500 μL of 0.5 M HNO₃, and then rinsed with 500 μL of MQ water (buffered to pH 7 with 2 % NH₄OH) 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 $\delta^{11}$B was also evaluated: : :” Please explain. This reads as if you used the internal carbonate standards to correct your $\delta^{11}$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?

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 $\delta^{11}$B of the external calcium carbonate standards JCp-1 (Porites sp.), NEP (Porites sp.) and JCl-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.

L308-322: Based on Table 3, it seems that batch separation with NH3 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.

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 $\delta^{11}$B values for these specimens are so different ($\sim 3.7$ 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, CaCO3 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 (>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 δ^{11}B (adapted from Rollion-Bard,2011b). The solid and dashed curves represent the δ^{11}B composition that would result from the incorporation of different amounts of B(OH)₃ into the marine carbonates. The dashed vertical lines represent the calculated pH based on the assumption that 0% B(OH)₃ is incorporated into temperature coral and 0%, 30% and 75% B(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 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 _11B variation as a function of pH” The theoretical model of carbonate _11B reflecting seawater pH has three parts: 1) borate _11B varies with pH in a known fashion (requires knowledge of pH, pKB* 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 _11B 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 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 _11B-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 _11B – 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 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 _11B-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 7-10. It currently shows the limits of sensitivity of the borate curve to pH, which is relevant given where the coralline algae data fall.
Reply to reviewer 3

We wish to thank the anonymous reviewer for their critical analysis of our manuscript and their helpful comments. We believe that we can address all of the major comments as indicated in the discussion below.

The manuscript by Sutton et al reports the boron isotope compositions of various marine calcifiers (coralline red alga, urchins, worm, coral, oyster). All the samples came from culture experiment (T=25 C, pCO2=409 μatm) and so should record the same _11B values if no vital effects are present. The _11B range of all the data is about 20‰ and seems to show the biological control on the calcification pH. I found the data interesting, but I think that there are a lot of repetitions through the text. Even if it is mentioned in the case of coralline red alga, the influence of B3 is not really taken into account. For example, the presence of B3 was also shown in corals, and it was not described in the text. In the figures, the symbols should be different between the calcium carbonate polymorphs.

Author response
We agree that we could expand the discussion on the influence of B3. We assume that in the case of corals, the reviewer is referring to Rollion-Bard et al. 2011b. We did not include a discussion for B incorporation in corals since, as the second reviewer pointed out “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)”. Although NMR gives evidence that trigonal boron is present in the calcite lattice, it cannot determine whether boric acid was in fact incorporated or if the trigonal boron originated from borate (see alternative mechanisms of boron incorporation in for example Klotchko, 2006; Noireaux et al., 2015).

However, we agree with the reviewers that we should expand our discussion within section 4.2 to provide more information on the different factors (including seawater pH and calcifying fluid pH) that can influence the speciation of boron and δ11B for inorganic calcite and aragonite. We will expand our discussion on this section to make our arguments more clear and include an extra figure (see attached Figure 5) with the following Figure caption: “The influence of pH on the speciation of boron and δ11B (adapted from Rollion-Bard, 2011b). The solid and dashed curves represent the δ11B composition that would result from the incorporation of different amounts of B(OH)3 into the marine carbonates. The dashed vertical lines represent the calculated pH based on the assumption that 0% B(OH)3 is incorporated into temperature coral and 0%, 30% and 75% B(OH)3 is incorporated into coralline alga.”

-L154: Please mention the study of Jorgensen et al (1985)

Author response
We will mention these studies.

-L108, 127: "2" must be in superscript
-L327: "range in range", please correct
Author response:
These will be changed

-section 4.3.3: It was already mentioned, please delete this section

Author response:
We think that this information is important to keep in the manuscript since it allows us to present a hypothesis that might explain the wide range of $\delta^{11}B_{\text{CaCO}_3}$ (20‰) observed for the species evaluated in this study. In addition, we would like to add a reference to table 4 to highlight the importance of this information: “Furthermore, there appears to be a moderate inverse relationship between the species’ relative ability to elevate calcification site pH and their empirically determined vulnerability to ocean acidification (Table 4).”

-L221: Interest for what? Why the data are not shown in the manuscript?

Author response:
The other elements (Ca, Na, Ba, U) are of interest to analyse since they can indicate whether the sample matrix has been washed out of the column. To be more clear, we will change this sentence to: Small aliquots of each sample were measured by single collector HR-ICPMS prior to analyses by MC-ICPMS to verify the retention of B on the column and removal of other elements (e.g. Ca, Na, Ba, U).

The B data are shown in the manuscript, see lines 275-278.

-L243-244: It was already mentioned, please delete

Author response:
I disagree, we did not state “Boron yields are evaluated by tracking B throughout the entire procedure.” prior to this sentence.

-L256: I suppose that there are older references than McCulloch et al (2014) for the MC-ICP-MS method.

Author response:
Yes, this is true. McCulloch et al. 2014 did a great job of describing the development of the MC-ICP-MS method for the analysis of B isotope analyses and in this case we felt it useful to cite a recent paper that summarizes the state of the art on method development. We will change the citation as follows: (see McCulloch et al, 2014 for up-to-date summary of methods).
- section 3.1.1.: Do you have an idea why the measurements on JCt-1 are more variable?

Author response:
The JCt-1 measurements in our study were variable for the different methods of sample injection (NH3 and d-DIHEN) but we did not see this variability for other samples or standards analysed with the same methods. We are not sure why the d-DIHEN method did not provide accurate results for JCt-1, but this has not influenced our conclusions. Further, the errors are still within acceptable limits as can be seen by the variability of the inter-laboratory study (lines 280-281).

-L288, 324: please add the errors on the _11B values

Author response:
We didn’t think it was necessary here since we are indicating the overall range in δ¹¹B values. We present the error bars related to the δ¹¹B of each species in the sentences that follow.

-L290: Why the error on the _11B value of the coralline alga is so high?

Author response:
As the reviewer noted, the intra-specific (same species but different organisms) reproducibility for the red coralline alga is large, however, the intra-organism (sub-sampling the same organism) and analytical reproducibility is not (see Table 3). This suggests that there is significant geochemical variability across the skeleton of this organism, but the analytical reproducibility is robust. We will make a more specific note of this in the text starting on line 289: “…and summarized in the text that follows. Note that the average data presented here (Table 4) represent intra-species reproducibility (i.e. measured differences between individual organisms of the same species), which can be substantial however, the intra-organism (sub-sampling of same organism) and analytical reproducibility (Table 3) are typical of single organism δ¹¹B analyses.”

-L334: No, in Noireaux et al (2015) there is a clear effect of the mineralogy (see figure 1)

Author response:
This is a glaring error on our part. We tried to simplify an argument, and the message was lost in translation. Thank you for picking up on this.

The sentence should read “Although Mavromatis et al. (2015) also found that polymorph mineralogy influences both the B/Ca ratio (higher in aragonite than calcite) and speciation of B in inorganic CaCO₃ (borate/boric acid ratio higher in aragonite than calcite), B incorporation alone does not appear to influence boron isotope fractionation.

-L370-371: What would be the pH of calcification if there is effectively 30% of B3? The _11B value of coralline alga could result from the combination of a pH increase and the incorporation of a certain proportion of B3.

Author response:
The reviewer asks an interesting question that can not be answered simply but does merit an extended discussion in the manuscript. Several related factors might influence the boron isotope composition of a calcifying organism including: the pH at the calcification site, the influence of pH on the speciation of B at the calcification site, boric acid incorporation into the calcite matrix, and the influence of boric acid on the trigonal structure of the lattice. Cusack et al. (2015) suggested that 30% of B in the calcite of a different coralline algae species was present in the trigonal B3 form; however, this does not necessarily suggest that the calcification fluid contained 30% boric acid or that 30% boric acid 70% borate was incorporated into the calcite lattice. Further empirical work is needed to clarify this relationship. However, if we were to ask the hypothetic scenario; what would be the pH at the calcification site be if 30% boric acid was available at the calcification site prior to biomineralization, we can answer that the calcification site pH would still be as high as 9, which is well above the ambient seawater pH of 8.1 (see table 4). As mentioned above, we will expand our discussion on this section to make our arguments clearer and include an extra figure (see attached Figure 5) with the following Figure caption: “The influence of pH on the speciation of boron and $\delta^{11}$B (adapted from Rollion-Bard, 2011b). The solid and dashed curves represent the $\delta^{11}$B composition that would result from the incorporation of different amounts of B(OH)$_3$ into the marine carbonates. The dashed vertical lines represent the calculated pH based on the assumption that 0% B(OH)$_3$ is incorporated into temperature coral and 0%, 30% and 75% B(OH)$_3$ is incorporated into coralline alga.”

-section 4.2.3: What are the calculated pH if the results of Noireaux et al (2015) for inorganic calcite are taken into account?

Author response:
We agree with the reviewers that we should expand our discussion within section 4.2 (as described previously in this response to reviewer) to provide more information on the different factors (including pH) that can influence the speciation of boron and $\delta^{11}$B for inorganic calcite and aragonite.

-L402: please remove ‘Notably....worm tubes’

Author response:
We will change this to: “To our knowledge these are the first reported B isotope measurements for worm tubes”

-L420: please remove ‘Notably....oysters’

Author response:
We will change this to: “To our knowledge these are the first reported B isotope measurements for oysters”

-L495: It is obvious. I do not see the point here.

Author response:
We thought it was important to clarify this point since it may not be obvious to all readers the extent to which $\alpha$ varied and our aim is to make the manuscript accessible to readers who may not all be very familiar with the boron isotope proxy so some basic statements like this can be
valuable.

Figure 1: Please use the alpha of Klochko et al (2006) and specify in the caption the pKa used and the alpha used.

Author response:

We will modify the figure attached above. The calculations are based on pKb = 8.1 (in seawater at 25 °C and a salinity of 35 under atmospheric), alpha=1.0272, d11Bsw=39.61

Figure 2: Please add data of Reynaud et al (2004), Lécuyer et al (2002), Farmer et al (2005). Please use the full name species of the foraminifera. 'Brachiopod' instead of 'Brochiopod';
We aim to show boron isotopic composition from the most studied marine biogenic carbonate archives including corals, foraminifera and bivalves. We also want to show that the data has been reported to 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 $^{11}$B-pH proxy in different species. For the above purpose, we will also replace the reference from Foster et al., 2008 to Holcomb et al., 2014, Sanyal et al., 1996 and Henehan et al., 2013.
In addition to the response to reviewers, we have also included a full listing of all relevant changes made in the manuscript (see below).

Changes are listed per page and line numbers refer to the marked up manuscript that follows.

Page 1-
L.14-38:
Inserted “boron,”
Deleted “of boron”;
Inserted “(δ_{11}B),
Deleted (B),
Inserted “of”
Deleted “in”
Inserted “skeletal”
Deleted (δ_{11}B)
Inserted “potentially critical”, removed “dominant”
Inserted “Bates, 2007; Feely et al., 2008; Dore et al., 2009; Byrne et al., 2010; Gonzalez-Davlia et al., 2010; Feely et al., 2016”
Inserted “marine”
Deleted “found”
Inserted “revealed”
Inserted “widely amonst”
Deleted “between”
Inserted “e.g.,”
Inserted “how an organism’s”
Inserted “its specific”
Deleted “the various”
Deleted “organismal”

Page 2
L.39-73
Deleted “Saenger et al., 2013”
Deleted “; Zinke et al., 2014”
Deleted “in time and space”
Inserted “(Zeebe and Wolf-Gladrow, 2001)”
Inserted “Hemming and Hanson, 1992”
Deleted “Pagani et al., 2005”
Deleted “noted”
Inserted “expressed”
Deleted “NIST, Gaithersburg, MD, USA”
Inserted “Catanzaro et al, 1970”
Deleted “0.04”
Deleted “fractionation factor between boric acid and borate ion in seawater (α)
Deleted “has been”
Inserted “was”

Page 3
L.74-102
“empirical species-specific calibrations between”
and/or potential isotopic fractionation during boron incorporation in biogenic carbonates, species-specific fractionation factors and transfer functions are likely more appropriate than theoretical α values
and seawater pH (pH$_{SW}$) are likely more appropriate than theoretical”
values if the goal is to reconstruct ambient seawater conditions”

Changed “The ⁸¹⁺¹⁻Β$_{CaCO₃}$ appears to be inherited from the ⁸¹⁺¹⁻Β composition of dissolved B(OH)$_₄$ in the solution from which that CaCO₃ precipitates, thereby quantitatively reflecting the pH of the precipitating solution. Experimental work (e.g., Hemming and Hanson, 1992; Sanyal et al., 2000) suggests that B(OH)$_₄$ is the dominant species of dissolved inorganic boron incorporated into CaCO₃ minerals as they precipitate from solution. It is also well established that ⁸¹⁺¹⁻Β of B(OH)$_₄$ is controlled by solution pH (c.f. Hemming and Hönisch, 2007; see discussion above). Therefore, the ⁸¹⁺¹⁻Β$_{CaCO₃}$ should reflect the pH of the precipitating solution. This, which has been demonstrated empirically in numerous studies (see Hemming and Hönisch, 2007, for summary). It should be noted that alternative models of boron incorporation into CaCO₃ have been proposed (Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015; Balan et al., 2016), although most published studies rely upon the framework first proposed by Hemming and Hanson (1992)” to
“The most widely applied framework in which the boron isotope composition of carbonates is interpreted relies on the assumption that B(OH)$_₄$ is the dominant species of dissolved inorganic boron incorporated into CaCO₃ minerals as they precipitate from solution. It is also well established that ⁸¹⁺¹⁻Β of B(OH)$_₄$ is controlled by solution pH (c.f. Hemming and Hönisch, 2007; see discussion above). Therefore, ⁸¹⁺¹⁻Β$_{CaCO₃}$ should reflect pH of the precipitating solution, which is consistent with the observations of a number of empirical studies (see Hemming and Hönisch, 2007, for summary). More recently alternative models of boron incorporation into CaCO₃ have been proposed (Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015; Balan et al., 2016). Generally speaking, these alternative models present a potential challenge to the utility of boron isotopes to reconstruct calcifying fluid and paleo-pH (Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015; Mavromatis et al., 2015; Balan et al., 2016). They present evidence consistent with boric acid, alongside borate, incorporation into some carbonates (eg. Noireaux et al., 2015; Uchikawa et al., 2015) and/or the presence trigonal boron in the carbonate lattice due to transformation from borate during precipitation (eg. Mavromatis et al., 2015). Some of these studies also highlight that calcite may be more prone to this complication than aragonite (e.g. Noireaux et al., 2015). Here, as an alternative hypothesis to a primary pH control over biomineral d11B composition, we also consider the compatibility of our data with the different models of boron incorporation.

L104-112 and onto page 4 to L122

Moved from page 4 “Many calcifying marine organisms, including scleractinian corals (Al-Horani et al., 2003; Cohen and Holcomb, 2009; Cohen and McConnaughey, 2003; Holcomb et al., 2010; Ries, 2011a), coralline red algae (Borowitzka and Larkum, 1987; McConnaughey and Whelan, 1997), calcareous green algae (De Beer and Larkum, 2001; Borowitzka and Larkum, 1987; McConnaughey and Falk, 1991), foraminifera (Rink et al., 1998; Zeebe and Sanyal, 2002), and crabs (Cameron, 1985) are thought to facilitate precipitation of their skeletal or shell CaCO₃ by elevating pH at their site of calcification. The effect of pH on CaCO₃ chemistry at the site of calcification can be summarized by the following equilibrium reactions:

H₂CO₃ ↔ HCO₃⁻ + H⁺
which are respectively governed by the following stoichiometric dissociation constants: 

\[ K^*1 = [\text{HCO}_3^-][\text{H}^+]/[\text{H}_2\text{CO}_3] \]

and

\[ K^*2 = [\text{CO}_3^{2-}][\text{H}^+]/[\text{HCO}_3^-] \]

Thus, reducing [\text{H}^+] at the site of calcification shifts the carbonic acid system towards elevated [\text{CO}_3^{2-}], thereby increasing CaCO_3 saturation state (\(\Omega\)CaCO_3) following:

\[ \Omega\text{CaCO}_3 = [\text{Ca}^{2+}][\text{CO}_3^{2-}]/K^*_{sp} \]

where K^*_{sp} is the stoichiometric solubility product of the appropriate CaCO_3 polymorph (e.g., calcite, aragonite, etc.) and is influenced by temperature and salinity.
Page 7

2.3 Materials

2.3.1 Samples

In this study, we evaluated the $\delta^{11}$B of 6 highly divergent species of marine calcifiers reared for 60 days in isothermal (25°C) and isosaline (32 practical salinity units; psu) seawater equilibrated with atmospheric pCO$_2$ of ca. 409 μatm, including a temperate coral (Oculina arbuscula), a coralline red alga (Neogoniolithon sp.), a tropical urchin (Eucidaris tribuloides), a temperate urchin (Arbacia punctulata), a serpulid worm (Hydroides crucigera), and an American oyster (Crassostrea virginica). Three modern marine carbonate standards were also analysed as part of our $\delta^{11}$B method validation, including: two corals (Porites sp.; JCp-1 from the Geological Survey of Japan; NEP, an internal standard from the University of Western Australia/Australian National University, McCulloch et al., 2014); and a giant clam (Tridacna gigas; JCt-1 from the Geological Survey of Japan).
monitored in this aliquot and the final solution in order to confirm complete removal of the sample matrix.

Inserted “; polypropylene”

Inserted “(see Section 2.4)”

Inserted “in individual tubes”

Inserted “(lowest yields for column = 0.5 ng and batch = 90 pg)”

Inserted “SRM”

Page 9
L.297-333

Inserted “, with”

Inserted “and the”

Deleted “did”

Inserted “of the external calcium carbonate standards”

Deleted “external”

Deleted “was also evaluated by analysing”

Inserted “are reported in the results section (see Section 3.1.1) alongside their published..”

Deleted “relative to”

Inserted “see.. for a recent summary of these methods”

Inserted “pHCF”

Deleted “calcification site pH”

Inserted “high efficiency”

Inserted “isotope”

Deleted “3.1.1 Yield and reproducibility”

Inserted “(represented as 2 standard deviations around the mean; 2SD)”

Page 10
L.334-370

Inserted “ laboratory”

Deleted “internal”

Deleted “3.1.2 Extraction blanks, boron recovery, and matrix removal”

Deleted “See sections 2.5 and 2.6. ”

Inserted “, and summarized in the text that follows. Note that the variance of the data presented in Table 4 represents inter-specimen variability (i.e., variability amongst different specimens of the same species), which is substantially greater than the intra-specimen variability (i.e., variability within a specimen) and analytical variability (variability amongst repeat analyses of the same subsample of a specimen) (Table 3).”

Deleted “organisms”

Inserted “species”

Inserted “pH_{SW}”

Deleted “seawater pH”

Inserted “pH_{SW}”

Deleted “seawater pH”

Page 11
L.-371-409

Changed “our” to “the”

Changed “d-DHIEN” to d-DIHEN” (throughout)
…, which is not consistent with prior observations for inorganic and organic calcite (e.g., Cusack et al., 2015, reported 30% trigonal B in the calcite lattice of a different species of coralline algae). Therefore, boric acid incorporation cannot alone rule out pH_{CF} as a potential driver of the anomalously elevated δ^{11}B_{CaCO3} observed here for coralline algae.). Moreover, although nuclear magnetic resonance spectroscopy reveals that trigonal boron is present in the calcite lattice, it cannot determine whether boric acid was incorporated directly into the calcite lattice, or if the trigonal boron originated from borate post-mineralization (e.g., see alternative mechanisms of boron incorporation discussed in Klochko, 2006; Noireaux et al., 2015). Nevertheless, if 30% of skeletal B is indeed directly incorporated into the calcite lattice of coralline algal skeleton, as reported by Cusack et al. (2015), pH_{CF} would still need to be as high as 9 to explain the anomalously high δ^{11}B_{CaCO3} (see Fig. 5). Short et al. (2015) observed that epiphytic turf algae can increase pH_{SW} up to 9 within the diffusive boundary layer above coralline algal crusts, driven by the algae’s photosynthetic drawdown of aqueous CO_{2}, lending support to the idea that coralline red algae could maintain their calcifying fluid near pH 9. Thus, δ^{11}B_{CaCO3} compositions of coralline red algae may indeed reflect substantially elevated pH_{CF} (9.4; Table 4, Fig. 4), suggesting that coralline red algae are highly efficient at removing protons and/or dissolved inorganic carbon from their calcifying medium.”
Inserted “pHCF”
Deleted “pH”

Inserted “theoretical value of δ11B for seawater borate”
Deleted “calculated δ11B CaCO3”
Deleted “calcification site pH”
Deleted “pH”

Inserted “Note that the δ11B values for these high-Mg calcite-precipitating organisms are also not consistent with significant boric acid incorporation into the carbonate lattice (Fig. 5).”
Modified “Serpulid worm” to “The serpulid worm”
Deleted “s secrete calcareous tubes with”
Deleted “being the only species that”
Inserted “their calcareous tube from a”
Deleted “In order to produce their calcareous tubes, H. crucigera”
Inserted “The worms initially”
Inserted “ultimately”
Inserted “SW”
Deleted “pH at its site of calcification”
Deleted “Notably, “To our knowledge
Inserted “To our knowledge

Changed “published δ11B CaCO3 data” to reported B isotope measurements”
Inserted “and the δ11B values for this mixed mineralogy precipitating organism is not consistant with significant boric acid incorporation into the carbonate lattice (Fig. 5).”
Deleted “Mollusks, such as

Page 14 and 15
L.488-526 and up to L.534

Deleted “pH”
Inserted “pHSW”
Inserted “pHCF”
Deleted “pH at the site of calcification”
Inserted “et al.,”
Changed “Fig. 6 to “Fig. 5”
Deleted “Notably, these are the first published δ11B CaCO3 data for”
Inserted “To our knowledge, these are the first reported B isotope measurements for oysters and the δ11B values for this low Mg calcite-precipitating organism are not consistent with significant boric acid incorporation into the carbonate lattice (Fig. 5).
Deleted “4.3 What does a species’ δ11B CaCO3 reveal about its calcification site pH and relative sensitivity to ocean acidification?”
Deleted “Understanding how marine organisms calcify is a critical requirement for understanding and, ideally, predicting their physiological responses to future ocean acidification (e.g., Kleypas et al., 2006).
Deleted “Notably, the temperature coral (O. arbuscula) and coralline red alga (Neogoniolithion sp.) have higher calculated calcification site pH (based on their boron isotope composition) than the other organisms. As discussed above (section 4.2), one possible explanation for these differences is that corals (and potentially coralline red algae) maintain their calcifying fluids at a higher pH than the calcifying fluids of other calcifying marine organisms.”
Moved to section 4.3.3 (from 4.3) “Notably, the different species’ δ11B CaCO3 and reconstructed calcification site pH appeared to exhibit a moderate, inverse relationship with their experimentally
determined vulnerability to ocean acidification (Ries et al., 2009). Species exhibiting more resilient ‘parabolic’ (e.g., coralline red alga) and ‘threshold’ (e.g., coral, tropical urchin) responses to ocean acidification generally exhibited a higher δ11BCaCO3 and, thus, calcification site pH than species exhibiting the more vulnerable ‘negative’ responses (e.g., oyster, serpulid worm) to ocean acidification (Table 4). The temperate urchin was the exception to this general trend, as it exhibited a relatively resilient parabolic response to ocean acidification yet maintained δ11BCaCO3 and, thus, calcification site pH close to that of seawater.

Moved within section 4.3 “In the absence of empirical measurements of calcifying fluid temperature, salinity, and δ11B, these parameters are generally assumed to reflect seawater.”

Deleted “However, the large variability in the calculated calcification site pH for these organisms (e.g. 7.9-9.4; Table 4) that were grown in near identical seawater conditions (pH = 8.0-8.2; Table 4) suggests that a biological process (e.g., regulation of calcification site pH) is governing boron isotope fractionation within the calcifying fluids and shells of marine calcifiers. Below,”

Moved down to section 4.3.1 “we evaluate the sensitivity of calculating pHCF from measured δ11BCaCO3 composition by testing the two principal factors that may influence the theoretical model of borate δ11B variation as a function of both pHCF and pHSW; namely pKB and α. A sensitivity analysis of δ11B in seawater was not conducted since all organisms evaluated in this study were exposed to seawater from the same source and, thus, of identical δ11B composition.

Deleted “identical δ11B composition.”

Page 15
L-535-562
Inserted “4.3 Estimating pHCF from δ11BCaCO3”

Inserted “The 6 species of calcifying marine organisms investigated in the present study exhibited average δ11BCaCO3 compositions ranging from 16.27 ‰ to 35.09 ‰ (Table 3)

Inserted “In the absence of empirical measurements of calcifying fluid temperature, salinity, and δ11B, these parameters are assumed to reflect seawater.”

Deleted “Notably, the temperature coral (O. arbuscula) and coralline red alga (Neogoniolithion sp.) have higher calculated pHCF (based on their boron isotope composition) than the other organisms.”

Moved up “4.3.1 Sensitivity of δ11BCaCO3

Inserted “based calculations of pHCF”

Inserted “to choice of pKB and α”

Deleted “for estimating seawater and calcification site pH”

Deleted “Other factors, such as the modification of seawater pH and incorporation of boric acid by marine calcifiers, are not presented here as they are discussed previously (see Section 4.2, Fig. 5). Also note that a”

Inserted “A sensitivity analysis of δ11B in seawater was not conducted since all organisms evaluated in this study were exposed to seawater from the same source and, thus, of identical δ11B composition.

Page 16
L.565-599
Inserted “CF”
Deleted “and the”

Inserted “δ11B of calcifying fluid (…CF),”
Deleted “of seawater”

Inserted “δ11BCaCO3”
Regardless of which $\alpha$ value is used, a wide range (ca. 20%) of $\delta^{11}\text{B}_{\text{CaCO}_3}$ compositions is observed amongst the marine calcifying species investigated. Furthermore, there appears to be a moderate inverse relationship between the species’ relative ability to elevate pHCFcalcification site pH and their empirically determined vulnerability to ocean acidification (see Ries et al., 2009 and Table 4). These results support the assertion that interspecific differences in pHCF calcification site pH contribute to marine calcifiers’ differential responses to ocean acidification – highlighting the need for future queries into the mechanisms driving boron isotope fractionation and biomineralization within marine calcifying organisms.

Understanding how marine organisms calcify is a critical requirement for understanding and, ideally, predicting their physiological responses to future ocean acidification (e.g., Kleypas et al., 2006).
Inserted “These results support the assertion that interspecific differences in pH<sub>CF</sub> calcification site pH contribute to marine calcifiers’ differential responses to ocean acidification – highlighting the need for future queries into the mechanisms driving boron isotope fractionation and biomineralization within marine calcifying organisms.”

Page 18
L653-675

Changed “borate δ<sup>11</sup>B” to “δ<sup>11</sup>B<sub>\text{\textsubscript{B(OH)}}</sub>”

Inserted “(see also Sanyal et al., 1996, Sanyal et al., 2001; Honisch et al., 2003; Trotter et al., 2011; Anagnostou et al., 2012)

Changed “calcification site pH” to “pH<sub>CF</sub>”

Deleted “pH at their site of calcification”

Inserted “pH<sub>CF</sub>”

References section

Inserted new literature cited


Deleted


Tables and Figures

Changed Table 4 description to:

“Summary of the average and standard deviation (SD) of δ11B for each species (‰), calculated pH of calcifying fluid (pH_{CF}), pH of seawater (pH_{SW}) during the experimental conditions, difference between pH_{CF} and pH_{SW} (ΔpH), calcification response to ocean acidification experiments (‘OA Response’; Ries et al., 2009), and shell/skeletal mineralogy (‘HMC’ = high-Mg calcite; ‘LMC’ = low-Mg calcite; Ries et al., 2009). In most cases 3 biological replicates of each species were analyzed. ‘NA’ = not available, only one biological replicate analysed. Note: SD is calculated from measurements of different individuals of the same species and this reflect interspecimen variability. Variability arising from intra-specimen variation (reflecting variability within a single specimen) and analytical error is provided in Table 3.”

Changed Table 5 description to “Previously published δ11B analyses of biogenic marine carbonates and seawater samples”

Changed Fig. 1. In response to reviewer 3 comments. Inserted new text into figure caption 1: “The pKb is 8.6 at 25 °C and 35 psu (Dickon, 1990), α is 1.0272 (Klochko et al., 2006), and δ11B_{SW} is 39.61 (Foster et al., 2010).”

Changed Fig. 2 in response to reviewers 2 and 3 (see reply to reviewer comments)
Changed figure caption 2: “8.6152” to “8.6”
Changed Fig. 4 in response to reviewer 2 comments (see reply to reviewer comments)

Changed in figure caption 4:
“with respect to” to “as a function of”
“borate δ¹¹B” to “δ¹¹B_{B(OH)₄}”
Deleted “fractionation factors”

Added new Figure in response to reviewer comments (Fig. 5)

Caption reads: “Exploring the potential influence of pH and boron speciation on carbonate δ¹¹B (adapted from Rollion-Bard et al., 2011b). The solid and dashed curves represent the δ¹¹B composition that would result from the incorporation of different amounts of B(OH)₃ into the biogenic carbonates. The dashed vertical lines represent the calculated pH based on the assumption that 0% B(OH)₃ is incorporated into the temperate coral skeleton and 0%, 30% and 75% B(OH)₃ is incorporated into the coralline algal skeleton. Of the calcite species examined, only the coralline algae has a δ¹¹B composition that could conceivably originate at least in part from B(OH)₃ incorporation.”
Abstract. The boron isotope composition of marine biogenic carbonates has been predominantly studied as a proxy for monitoring past changes in seawater pH and carbonate chemistry. In order to derive seawater pH from boron isotope ratio data, a number of assumptions related to chemical kinetics and thermodynamic isotope exchange reactions are necessary. Furthermore, the boron isotope composition of biogenic carbonates is assumed to reflect the δ11B of dissolved borate (B(OH)₄⁻) in seawater. Here we report the development of methodology for measuring the δ11B in biogenic carbonate samples at the multi-collector inductively coupled mass spectrometry facility at Ifremer (Plouzané, France) and the evaluation of δ11B in a diverse range of marine calcifying organisms. We evaluated the δ11B of 6 species of marine calcifiers (a temperate coral, Oculina arbuscula; a coralline red alga, Neogoniolithion sp.; a tropical urchin, Eucidaris tribuloides; a temperate urchin, Arbacia punctulata; a serpulid worm, Hydroides crucigera; and an American oyster, Crassostrea virginica) that were reared for 60 days in isothermal seawater (25°C) equilibrated with an atmospheric pCO₂ of ca. 409 µatm. We observe large inter-species variability in δ11B (ca. 20 ‰) and significant discrepancies between measured δ11B and expected from established relationships between δ11B and seawater pH. We discuss these results in the context of various proposed mechanisms of biocalcification, including the potential dominant role that internal calcifying site pH plays in regulating CaCO₃ saturation state and borate δ11B at the site of calcification and, thus, the δ11B composition of calcifiers’ shells and skeletons.

1 Introduction

The ability to monitor historical changes in seawater pH on both short and long-term timescales is necessary to understand the influence that dramatic changes in atmospheric CO₂ (pCO₂) have on marine carbonate chemistry. The recent anthropogenic increase in pCO₂ has already resulted in a significant decrease in seawater pH (Bates, 2007; Feely et al., 2008; Dore et al., 2009; Byrne et al., 2010; Gonzalez-Davila et al., 2010; IPCC, 2014; Feely et al., 2016), with potential effects on the ability of marine calcifying organisms to produce skeletal calcium carbonate (CaCO₃; IPCC, 2014). Ocean acidification studies have found that organismal responses vary widely between taxa, highlighting the complexity of biological responses to ocean acidification (e.g., Ries et al., 2009; Kroeker et al., 2010; Kroeker et al., 2013) and necessitating a more complete understanding of how an organism’s various mechanisms of biocalcification govern its specific responses to ocean acidification.
1.1 Theoretical model of $\delta^{11}B$ variation with pH

The boron isotope composition ($\delta^{11}B$) of biogenic CaCO$_3$ ($\delta^{11}B_{\text{CaCO}_3}$) has been primarily used as a palaeoceanographic proxy for seawater pH (Hönisch and Hemming, 2004; Hönisch et al., 2004; Montagna et al., 2007; Palmer, 1998; Pearson et al., 2009; Penman and Hönisch, 2014; Rae et al., 2011; Saenger et al., 2013; Trotter et al., 2011; Vengosh et al., 1991; Wei et al., 2009; Zinke et al., 2014). Boron has a residence time in seawater of ca. 14 million years (Lemarchand et al., 2000), which is much longer than the mixing time of oceans (ca. 1000 years), suggesting that it behaves conservatively in time and space (Foster et al., 2010), making $\delta^{11}B$ an attractive palaeo-proxy for pH. The development of this proxy is based on a theoretical model of $\delta^{11}B$ variation with pH (Zeebe and Wolf-Gladrow, 2001) that assumes that $\delta^{11}B_{\text{CaCO}_3}$ reflects the $\delta^{11}B$ composition of borate in seawater ($\delta^{11}B_{\text{BSW(OH)}_4}$; Hemming and Hanson, 1992Pagani et al., 2005). The theoretical model of $\delta^{11}B$ variation as a function of pH requires knowledge of the fractionation factor ($\alpha$) for isotope exchange between the aqueous species of boron, the dissociation constant ($pK_B$), and the isotopic composition of boron in seawater (Pagani et al., 2005).

Boron exists in aqueous solutions as either trigonal boric acid [B(OH)$_3$] or as the tetrahedral borate anion [B(OH)$_4^-$] and their proportions in solution are pH dependent (Fig. 1), as defined by the following equilibrium reaction:

$$\text{B(OH)}_3 + \text{H}_2\text{O} \rightleftharpoons \text{B(OH)}_4^- + \text{H}^+$$

In modern seawater, B(OH)$_4^-$ represents $\sim24.15$% of boron species, assuming that the dissociation constant ($pK_B$) between these two species of boron is 8.597 (at 25 °C, pH = 8.1; Dickson, 1990). Boron has two stable isotopes ($^{10}B$ and $^{11}B$) with relative abundances of 19.9% and 80.1%, respectively, and B(OH)$_3$ is enriched in $^{11}B$ relative to B(OH)$_4^-$ due to molecular differences of these chemical species in solution. The isotopic composition of boron is noted expressed following standard convention:

$$\delta^{11}B = \left[\frac{(^{11}B_{\text{sample}}/^{10}B_{\text{sample}})}{(^{11}B_{\text{standard}}/^{10}B_{\text{standard}})}\right] \times 1000 \text{‰}$$

where the reference standard is NIST SRM 951 (NIST, Gaithersburg, MD, USACatanzaro et al, 1970).

The $\delta^{11}B$ of modern seawater is 39.61 ± 0.04 20‰ (Foster et al., 2010) and a large (> 20‰) and constant fractionation factor of $\delta^{11}B$ exists between the two aqueous species described above. The $\alpha$ fractionation between boric acid and borate ion is defined as:

$$\alpha = \frac{\text{Boric acid}}{\text{borate ion}}$$

A wide range of theoretical and empirical values for the $\alpha$ fractionation factor between boric acid and borate ion in seawater (\(\alpha\)) have been suggested (Byrne et al., 2006; Kakihana et al., 1977; Klochko et al., 2006; Nir et al., 2015; Palmer et al., 1987). For example, $\alpha = 1.0194$ was calculated from theory by Kakihana et al. (1977) and has been widely applied in palaeo-reconstructions of seawater pH (Hönisch et al., 2004; Kakihana et al., 1977; Sanyal et al., 1995). Zeebe (2005) used analytical techniques and \textit{ab initio} molecular orbital theory to calculate $\alpha$ ranging from 1.020 to 1.050 at 300 K. Zeebe (2005) provided several arguments in support of $\alpha \geq 1.030$, ultimately concluding that experimental work was required to determine the $\alpha$ between dissolved boric acid and the borate ion. Subsequent to the work by Zeebe (2005), significant error was identified for the borate vibrational spectrum term used in Kakihana et al.’s (1977) theoretical calculation of $\alpha$ (Klochko et al., 2006; Rustad and Bylaska, 2007). An empirical $\alpha$ of 1.0272 (Klochko et al., 2006), using a corrected borate vibrational spectrum term, is now considered to best describe the boron isotope fractionation between dissolved boric acid and borate ion in seawater.
Moreover, due to the ability of some calcifying organisms to alter carbonate chemistry at their site of calcification, empirical species-specific calibrations between $\delta^{11}B_{\text{CaCO}_3}$ and/or potential isotopic fractionation during boron incorporation in biogenic carbonates, species-specific fractionation factors and transfer functions are likely more appropriate than theoretical $\alpha$ values if the goal is to reconstruct ambient seawater conditions (Anagnostou et al., 2012; Hönisch et al., 2004; Krief et al., 2010; Rae et al., 2011; Reynaud et al., 2004; Trotter et al., 2011).

The $\delta^{11}B_{\text{CaCO}_3}$ appears to be inherited from the $\delta^{11}B$ composition of dissolved $\text{B(OH)}_4^-$ in the solution from which that $\text{CaCO}_3$ precipitates, thereby quantitatively reflecting the pH of the precipitating solution. Experimental work (e.g., Hemming and Hanson, 1992; Sanyal et al., 2000) suggests that $\text{B(OH)}_4^-$ is the dominant species of dissolved inorganic boron incorporated into $\text{CaCO}_3$ minerals as they precipitate from solution. It is also well established that $\delta^{11}B$ of $\text{B(OH)}_4^-$ is controlled by solution pH (e.g. Hemming and Hönisch, 2007; see discussion above). Therefore, the $\delta^{11}B_{\text{CaCO}_3}$ should reflect the pH of the precipitating solution. This, which has been demonstrated empirically in numerous studies (see Hemming and Hönisch, 2007, for summary).

It should be noted that alternative models of boron incorporation into $\text{CaCO}_3$ have been proposed (Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015; Balan et al., 2016), although most published studies rely upon the framework first proposed by Hemming and Hanson (1992). The most widely applied framework in which the boron isotope composition of carbonates is interpreted relies on the assumption that $\text{B(OH)}_4^-$ is the dominant species of dissolved inorganic boron incorporated into $\text{CaCO}_3$ minerals as they precipitate from solution. It is also well established that $\delta^{11}B$ of $\text{B(OH)}_4^-$ is controlled by solution pH (e.g. Hemming and Hönisch, 2007; see discussion above). Therefore, $\delta^{11}B_{\text{CaCO}_3}$ should reflect pH of the precipitating solution, which is consistent with the observations of a number of empirical studies (see Hemming and Hönisch, 2007, for summary).

More recently alternative models of boron incorporation into $\text{CaCO}_3$ have been proposed (Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015; Balan et al., 2016). Generally speaking, these alternative models present a potential challenge to the utility of boron isotopes to reconstruct calcifying fluid and paleo-pH (Klochko et al., 2009; Noireaux et al., 2015; Mavromatis et al., 2015; Balan et al., 2016). They present evidence consistent with boric acid, alongside borate, incorporation into some carbonates (e.g. Noireaux et al., 2015; Uchikawa et al., 2015) and/or the presence trigonal boron in the carbonate lattice due to transformation from borate during precipitation (e.g. Mavromatis et al., 2015). Some of these studies also highlight that calcite may be more prone to this complication than aragonite (e.g. Noireaux et al., 2015). Here, as an alternative hypothesis to a primary pH control over biomineral $\delta^{11}B$ composition, we also consider the compatibility of our data with the different models of boron incorporation.

### 1.2 The role of calcification site pH in calcareous biomineralization and organisms’ responses to ocean acidification

Many calcifying marine organisms, including scleractinian corals (Al-Horani et al., 2003; Cohen and Holcomb, 2009; Cohen and McConnaughey, 2003; Holcomb et al., 2010; Ries, 2011a), coralline red algae (Borowitzka and Larkum, 1987; McConnaughey and Whelan, 1997), calcareous green algae (De Beer and Larkum, 2001; Borowitzka and Larkum, 1987; McConnaughey and Falk, 1991), foraminifera (Rink et al., 1998; Zeebe and Sanyal, 2002), and crabs (Cameron, 1985) are thought to facilitate precipitation of their skeletal or shell $\text{CaCO}_3$ by elevating pH at their site of calcification. The effect of pH on $\text{CaCO}_3$ chemistry at the site of calcification can be summarized by the following equilibrium reactions:

\[
\text{H}_2\text{CO}_3 \leftrightarrow \text{HCO}_3^- + \text{H}^+
\]

and

\[
\text{H}_2\text{CO}_3 \leftrightarrow \text{H}_2\text{O} + \text{CO}_2
\]
\[
\text{HCO}_3^- \leftrightarrow \text{H}^+ + \text{CO}_3^{2-};
\]

which are respectively governed by the following stoichiometric dissociation constants:

\[
K^*_{1} = \frac{[\text{HCO}_3^-][\text{H}^+]}{[\text{H}_2\text{CO}_3]}
\]

and

\[
K^*_{2} = \frac{[\text{CO}_3^{2-}][\text{H}^+]}{[\text{HCO}_3^-]}
\]

Thus, reducing [H\(^+\)] at the site of calcification shifts the carbonic acid system towards elevated [CO\(_3^{2-}\)], thereby increasing CaCO\(_3\) saturation state (\(\Omega_{\text{CaCO}_3}\)) following:

\[
\Omega_{\text{CaCO}_3} = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]}{K^*_{\text{sp}}}
\]

where \(K^*_{\text{sp}}\) is the stoichiometric solubility product of the appropriate CaCO\(_3\) polymorph (e.g., calcite, aragonite, etc.) and is influenced by temperature and salinity.

The decrease in seawater pH\(_{\text{SW}}\) that will accompany the forecasted rise in anthropogenic atmospheric pCO\(_2\) will reduce seawater [CO\(_3^{2-}\)], which has been shown to inhibit biological deposition of CaCO\(_3\), or even promote its dissolution (c.f. Doney et al., 2009; Fabry et al., 2008; Kleypas et al., 2006; Kroeker et al. 2010; Langdon, 2002; Ries et al., 2009). However, if seawater is the source of an organism’s calcifying fluid (e.g., Gaetani and Cohen, 2006), then the concentration of dissolved inorganic carbon (DIC) in this fluid will increase as atmospheric pCO\(_2\) increases. Organisms able to strongly regulate \(\text{pH}\) of their calcifying fluid (\(\text{pH}_{\text{CF}}\)), despite reduced external \(\text{pH}\), should convert much of this increased DIC, occurring primarily as HCO\(_3^-\), back into the CO\(_3^{2-}\) that they need for calcification (Ries, 2011a, 2011b; Ries et al., 2009). Thus, an organism’s specific response to CO\(_2\)-induced ocean acidification is critically dependent upon that organisms’ ability to maintain an elevated \(\text{pH}\) at their site of calcification.

It should be noted that marine calcifiers biomineralize in diverse ways, and that some calcifers’ mechanisms of biomineralization are better understood than others. Corals are thought to accrete CaCO\(_3\) directly from a discrete calcifying fluid (e.g., Cohen and McConnaughey, 2003 and references therein; Al-Horani et al., 2003; Cohen and Holcomb, 2009; Gaetani and Cohen, 2006; Ries, 2011a), with mineralization sites and crystal orientations being influenced by organic templates and/or calicoblastic cells (e.g., Cuif and Dauphin, 2005; Goldberg, 2001; Melborn et al., 2008; Tambuté et al., 2007). Mollusks are also thought to precipitate their shells from a discrete calcifying fluid between the external epithelium of the mantle and the inner layer of the shell known as the extrapallial fluid (e.g., Crenshaw, 1972), with hemocytes and organic templates playing a potentially important role in crystal nucleation (e.g., Mairie et al., 2012; Mount et al., 2004; Weiner et al., 1984). Coralline red algae, such as those belonging to the family Corallinaceae, are also thought to precipitate high-Mg calcite (and/or aragonite) crystals from an intercellular calcifying fluid (Simkiss and Wilbur, 1989). Notably, biomineralization by coralline red algae occurs primarily within the cell wall and often has a preferred crystal orientation, which is not typical of other calcifying macroalgae (Simkiss and Wilbur, 1989). Echinoids, in contrast, are thought to initiate calcification on Ca\(^{2+}\)-binding organic matrices within cellular vacuoles (Ameye et al., 1998). Many calcifying marine organisms, including scleractinian corals (Al-Horani et al., 2003; Cohen and Holcomb, 2009; Cohen and McConnaughey, 2003; Holcomb et al., 2010; Ries, 2011a), coralline red algae (Borowitzka and Larkum, 1987; McConnaughey and Whelan, 1997), calcareous green algae (De Beer and Larkum, 2001; Borowitzka and Larkum, 1987; McConnaughey and Fall, 1991), foraminifers (Rink et al., 1998; Zeebe and Sanyal, 2002), and crabs (Cameron, 1985) are...
thought to facilitate precipitation of their skeletal or shell CaCO$_3$ by elevating pH at their site of calcification. The effect of pH on CaCO$_3$ chemistry at the site of calcification can be summarized by the following equilibrium reactions:

$$\text{H}_2\text{CO}_3 \rightleftharpoons \text{HCO}_3^- + \text{H}^+$$

and

$$\text{HCO}_3^- \rightleftharpoons \text{H}^+ + \text{CO}_3^{2-}$$

which are respectively governed by the following stoichiometric dissociation constants:

$$K_1^* = [\text{HCO}_3^-][\text{H}^+] / [\text{H}_2\text{CO}_3]$$

and

$$K_2^* = [\text{CO}_3^{2-}][\text{H}^+] / [\text{HCO}_3^-]$$

Thus, reducing [H$^+$] at the site of calcification shifts the carbonic acid system towards elevated [CO$_3^{2-}$], thereby increasing CaCO$_3$ saturation state (Ω$_\text{CaCO}_3$) following:

$$Ω_{\text{CaCO}_3} = [\text{Ca}^{2+}][\text{CO}_3^{2-}] / K_\text{sp}^*$$

where $K_\text{sp}^*$ is the stoichiometric solubility product of the appropriate CaCO$_3$ polymorph (e.g., calcite, aragonite, etc.).

Various mechanisms have been proposed for elevating pH$_\text{CF}$ at the site of calcification, including conventional H$^+$-channeling (McConnaughey and Falk, 1991), Ca$^{2+}$-H$^+$ exchanging ATPase (Cohen and McConnaughey, 2003; McConnaughey and Falk, 1991; McConnaughey and Whelan, 1997), light-induced H$^+$-pumping (De Beer and Larkum, 2001), transcellular symporter and co-transporter H$^+$- solute shuttling (McConnaughey and Whelan, 1997), cellular extrusion of hydroxyl ions (OH$^-$) into the calcifying medium, and CO$_2$-consumption via photosynthesis (e.g., Borowitzka and Larkum, 1976).

Regardless of the exact composition (e.g., seawater vs. modified seawater) or nature (e.g., fluid vs. gel) of their calcifying media, or the specific mechanisms by which they produce their CaCO$_3$ (e.g., organic templates vs. cellular mediation vs. proton-pumps vs. Ca$^{2+}$-ATPase), an organism’s ability to control pH$_\text{CF}$ at their site of calcification should strongly influence their ability to convert DIC into CO$_3^{2-}$, thereby impacting their specific calcification response to CO$_2$-induced ocean acidification.

### 1.43 Relationship between calcification site pH and δ$^{13}$B$_\text{CaCO}_3$

Organisms that precipitate CaCO$_3$ from a discrete calcifying fluid should record in their shells and skeletons δ$^{13}$B$_\text{CaCO}_3$ compositions that reflect pH$_\text{CF}$ of their calcifying fluid (McCulloch et al., 2012; Holcomb et al., 2014; Farmer et al., 2015; Martin et al., 2016). Numerous studies have documented a relationship between the pH$_\text{SW}$ pH of seawater and the δ$^{13}$B$_\text{CaCO}_3$ composition of foraminiferal shells and coral skeletons (Fig. 2). However, the observed relationships between biogenic δ$^{13}$B$_\text{CaCO}_3$ and pH$_\text{SW}$ seawater pH vary widely amongst taxa (Fig. 2), and generally differ from that measured or derived theoretically for B(OH)$_4^-$ in seawater (Byrne et al., 2006; Klochko et al., 2006; Liu and Tossell, 2005; Zeebe, 2005) and from that observed in abiotically precipitated CaCO$_3$ (Sanyal et al., 2000; Noireaux et al., 2015).

One hypothesis for the discrepancies between the expected δ$^{13}$B$_\text{CaCO}_3$-pH relationship and that actually observed for biogenically precipitated CaCO$_3$ exists because most marine calcifiers are not precipitating their CaCO$_3$ directly from
seawater, but rather from a discrete calcifying fluid with a pH that is substantially elevated relative to that of their external seawater. Prior studies have shown that, for a given pH$_{sw}$ seawater-pH, δ$^{11}$B$_{CaCO_3}$ of the coral species *Porites cylindrica* and *Acropora nobilis* is moderately elevated relative to δ$^{11}$B$_{CaCO_3}$ of the foraminifera *Globigerinoides sacculifer* and substantially elevated relative to the mollusk *Mytilus edulis* (Fig. 2; Heinemann et al., 2012; Hönsch et al., 2004; Sanyal et al., 2001). One possible explanation for these differences is that corals are maintaining their calcifying fluids at higher pH than the calcifying fluids of foraminifera, which are in turn elevated relative to the pH$_{cy}$ calcifying fluid pH of mussels. This is consistent with pH microelectrode (Al-Horani et al., 2003; Ries, 2011a), boron isotope (e.g., Rollion-Bard et al., 2003, Rollion-Bard et al., 2011b; Anagnostou et al., 2012; Krief et al., 2010; Trotter et al., 2011; McCulloch et al., 2012; Wall et al., 2016), and fluorescent pH dye data (Venn et al., 2009, 2011, 2013), suggesting that scleractinian corals elevate their pH$_{cy}$ calcifying fluid pH to 8.5 – 10, versus their external pH$_{sw}$ seawater-pH of 8, that foraminifera maintain their pH$_{cy}$ calcifying fluid pH between 8 and 9 (Jorgensen et al., 1985; Rink et al., 1998), and that bivalves maintain their pH$_{cy}$ extrapallial fluid pH between 7.5 and 8 (Crenshaw, 1972).

Here, we investigate differences in δ$^{11}$B$_{CaCO_3}$-pH relationships amongst taxonomically diverse biogenic calcification systems and discuss the compatibility of these observations with the hypothesis that δ$^{11}$B$_{CaCO_3}$ of biogenic carbonate is recording pH$_{cy}$ of the organisms’ calcifying fluids (rather than pH$_{sw}$ the organism’s surrounding seawater) — a key parameter of biological calcification that has proven challenging to measure yet is fundamental to understanding, and even predicting, marine calcifiers’ responses to CO$_2$-induced ocean. By systematically investigating the δ$^{11}$B$_{CaCO_3}$ composition of a taxonomically broad range of taxa, each employing different mechanisms of calcification, yet all cultured under equivalent laboratory conditions (Ries et al., 2009), we are able to empirically assess biological controls on the δ$^{11}$B$_{CaCO_3}$ composition of biogenic carbonates.

In this study, we evaluated the δ$^{11}$B$_{CaCO_3}$ of 6 highly divergent species of marine calcifiers reared for 60 days in isothermal (25°C) and isosaline (32 practical salinity units; psu) seawater equilibrated with atmospheric pCO$_2$ of ca. 409 µatm, including a temperate coral (*Oculina arbuscula*), a coraline red alga (*Neogoniolithion sp*.), a tropical urchin (*Eucidaris tribuloides*), a temperate urchin (*Arbacia punctulata*), a serpulid worm (*Hydroides crucigera*), and an American oyster (*Crassostrea virginica*). Three modern marine carbonate standards were also analysed as part of our δ$^{11}$B$_{CaCO_3}$ method validation, including: two corals (*Porites sp. ICP-1 from the Geological Survey of Japan; NEP, an internal standard from the University of Western Australia/Australian National University, McCulloch et al., 2014), and a giant clam (*Tridacna gigas; JCt-1 from the Geological Survey of Japan*).

2 Methods and materials

2.1 Laboratory conditions

Sample processing and chemical separation were performed under ISO 5 (class 100) laminar flow hoods within an ISO 6 (class 1,000) clean room at Ifremer (Plouzané, France). Analyses of $^{11}$B/$^{10}$B ratios were carried out using a Thermo Scientific Neptune MC-ICP-MS at the Pôle Spectrométrie Océan (PSO), Ifremer (Plouzané, France). Efforts were made to minimize sample exposure to laboratory air.

2.2 Reagents

Ultra-pure reagents were used for all chemical procedures. The source of high-purity water (UHQ) for the procedure is a Millipore Direct-Q water purification system with a specific resistivity of 18.2 MΩ cm. All HNO$_3$ solutions are obtained from dilutions using Aristar Ultra-high purity acid. The 0.5 N NH$_4$OH solutions are boron-cleaned by exchange with boron-specific resin (Amberlite IRA 743). UHQ water is buffered to pH 7 with the boron-cleaned NH$_4$OH. The reagent boron blanks were
measured on a Thermo Scientific Element XR at the PSO, Ifremer (Plouzané, France) and were all < 0.1 ppb, yielding a total B blank of <100 ng per sample.

2.3 Materials

2.3.2 Samples

In this study, we evaluated the $\delta^{11}B$ of 6 highly divergent species of marine calcifiers that had been reared for 60 days in isothermal (25°C) and isotonic (32 practical salinity units; psu) seawater equilibrated with atmospheric pCO$_2$ of ca. 409 μatm, including a temperate coral (Oculina arbuscula), a coralline red alga (Neogoniolithon sp.), a tropical urchin (Eucidaris tribuloides), a temperate urchin (Arbacia punctulata), a serpulid worm (Hydroides crucigera), and an American oyster (Crassostrea virginica; see Ries et al., 2009 for details). The samples were subsampled for new growth relative to a barium marker emplaced at the start of the experiment (details in Ries, 2011), homogenized, and multiple specimens per taxa (same species, multiple organisms) were evaluated (as indicated under the sub-title “Name” 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.

2.3.2 Standards

A range of standards were used in this study, including: (1) the reference standard NIST SRM 951 (Catanzaro et al., 1970; Gaithersburg, MD, USA) for B isotope ratio and B concentration; (2) a mixture of NIST SRM 951 and a series of ICPMS SRM for B:Ca ratio (30-200 μg/mg); (3) the international coral standard (Porites sp.) JCp-1 (Geological Survey of Japan, Tsukuba, Japan); (4) the international giant clam standard (Tridacna gigas) JCt-1 (Geological Survey of Japan, Tsukuba, Japan); and (5) NEP a n internal laboratory coral standard (NEP; Porites sp.) NEP from University of Western Australia/Australian National University (McCulloch et al., 2014).

2.4 Boron extraction procedure

Prior to boron isotope analysis, B was separated from the sample matrix using a B-specific anionic exchange resin (Amberlite IRA-743; Kiss, 1988). Amberlite IRA 743 behaves as an anion exchanger with a high affinity for B absorption at neutral to alkaline pH (i.e., will absorb B), and a low affinity for boron at acidic pH (i.e., will release B). 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).

Here, we present two methods of B extraction (batch and column chemistry), where the influence of matrix chemistry is removed through minor adjustments to the chemistry of existing B extraction techniques. These two methods were applied to various biogenic CaCO$_3$ samples (Porites coral, temperate urchin, giant clam, American oyster). All samples are cleaned with an oxidative cleaning method (described in detail below) using NH$_4$OH-buffered H$_2$O$_2$ followed by multiple washes with pH-buffered UHQ water (pH = 7).

2.4.1 Oxidative cleaning

Samples and reference materials JCp-1, JCt-1, and NEP were cleaned with an oxidative cleaning method following the method of Barker et al. (2003). For a 2 mg sample, 200 μL of the alkaline-buffered (0.1 M NH$_4$OH) H$_2$O$_2$ was added to remove organic matter. Samples were placed in an ultrasonicator for 20 minutes at 50°C to expedite cleaning. Following peroxide cleaning, samples were then submitted to multiple washes (typically 3) of UHQ water (pH = 7, 400 μL) until the pH of the supernatant matched that of the UHQ water to ensure removal of all oxidizing agent. The water was then removed from samples after centrifugation and a weak-acid leach was implemented by adding 20 μL of 0.001 M HNO$_3$ to each sample. Samples were then ultrasonicated for 10 minutes, centrifuged, and then before the acid was removed, The samples were washed twice, and
replaced with pH-buffered UHQ water, centrifuged, and the water was removed. Dissolution of each sample was then performed by addition of 20 μL of 3 M HNO₃ followed by 300 μL of 0.05 M HNO₃. The pH of each sample was then adjusted to pH 7 with 0.2 M NH₄OH, following partition coefficients for the B-specific resin reported by Lemarchand et al. (2002). For both the batch and the column chemistry methods, the resin is pre-cleaned and conditioned to a pH of 7 prior to sample loading.

2.4.2 Column chemistry method

A column chemistry protocol for B extraction (described in Table 1) was developed based on methods described by Wang et al. (2010) and Foster et al. (2013). Briefly, the columns were washed with pH-buffered MQ-H₂O (pH=7), 0.5 M HNO₃, and again with pH-buffered MQ-H₂O. The eluent was measured to ensure that it was at pH 7 prior to loading of the sample. The sample was then loaded onto the resin and washed multiple times (1500 μL x 3) with pH-buffered UHQ in order to remove any cations, and then the B was eluted in 1000 μL of 0.5 M HNO₃. Column yields were greater than 95 % (Fig. 3) and elution tails of every sample were checked with an extra 500 μL acid rinse. In all cases, this tail represented less than 1 % of B loaded. Small aliquots of each sample were measured by single collector HR-ICPMS prior to analyses by MC-ICPMS to verify the retention of B on the column and removal of other elements (e.g. Ca, Na, Ba, U). Boron concentrations of small aliquots of each sample were measured by single collector ICPMS prior to analysis, and the concentration of other elements of interest (Ca, Na, Ba, U) were also monitored in this aliquot and the final solution in order to confirm complete removal of the sample matrix.

2.4.3 Batch method

The batch method approach to B separation was conducted under closed conditions in an attempt to reduce airborne B contamination. Cleaned samples (pH 7) were transferred into acid-cleaned microcentrifuge tubes (500 μL; polypropylene) containing 5 mg of resin (see Section 2.4), which is B-cleaned in individual tubes with 500 μL of 0.5 M HNO₃, and then rinsed with 500 μL of MQ water (buffered to pH 7 with 2 % NH₄OH) three times to elute the other cations in the matrix and achieve pH 7. Tubes were then capped and shaken for 15 minutes to promote exchange of anions from the aqueous sample to the resin. Afterwards, the mixture was centrifuged (1 min, 2000 rpm), the matrix was decanted, and the resin was washed three times (200 μL) with pH-buffered (pH 7) UHQ water to elute any cations. Boron recovery was then performed with the addition of 500 μL of 0.05 M HNO₃ and shaken again for 15 min to promote the anion exchange between the resin and solution. A final tail-check was performed with 100 μL of 0.05 M HNO₃ to ensure that all of the B was recovered in the initial 500 μL 0.05 M HNO₃ solution.

2.5 Procedural blanks

The total yield of B from procedural blanks, which encompasses reagent, air-borne and procedural contamination, was sub-nanogram (lowest yields for column = 0.5 ng and batch = 90 pg). Such low contamination was achieved through stringent cleaning and handling protocols for all consumables and reagents, thereby permitting accurate measurement of B at sub-μM concentrations.

2.6 Boron recovery and matrix removal

A major challenge in the measurement of δ¹¹B by MC-ICPMS is the elimination of residual boron from prior analyses (i.e., ‘memory effects’). In order to evaluate memory effects, multiple concentrations (30 ppb to 130 ppb) of a standard solution (NIST SRM 951) were analysed. After washing out the MC-ICPMS with a solution of 0.05 M HNO₃ for several minutes, the residual ¹¹B and ¹⁰B signals were in the range of 10 – 80 mV, equivalent to 5 % (30 ppb) and 3 % (130 ppb), respectively (see Fig. S1 for ¹¹B blanks). Boron recovery was measured using a Thermo Scientific Element XR HR-ICP-MS at the Laboratory
for Geochemistry and Metallogeny, Ifremer (Plouzané, France). Boron yields are evaluated by tracking B throughout the entire procedure.

2.7 Mass spectrometry

Isotopic measurements were conducted using a Thermo Scientific Neptune MC-ICPMS at the PSO, Ifremer (Plouzané, France), operated with standard plasma settings. To account for drift in mass discrimination through the analysis, samples were bracketed by matrix-matched standards of similar composition. Typically, the concentration of the standard (NIST SRM 951) was 50 ppb in 0.05 M HNO₃. Each analysis consisted of a 2-minute simultaneous collection of masses 11 and 10 on Faraday cups H3 and L3 equipped with 10¹¹ Ω resistors. Each sample was analysed in duplicate during a single analytical session, with the replicate analyses did not sharing a bracketing standard. As such, the boron isotope ratios are determined as delta values (δ¹¹B). The δ¹¹B of the external calcium carbonate standards was also evaluated by analysing JCp-1 (Porites sp.), NEP (Porites sp.) and JCT-1 (hard clam) standards, which were processed in the same manner and are reported in the results section (see Section 3.1.1) alongside relative to their published reference values (Foster et al., 2013; McCulloch et al., 2014).

The MC-ICPMS method is a commonly used approach to measure δ¹¹B due to its capacity for rapid, accurate and reproducible analyses (see McCulloch et al., 2014, for a recent up-to-date summary of these methods). Challenges with this method arise from the volatile and persistent nature of boron that can result in significant memory effects, cross-contamination between samples and standards, and unanticipated matrix effects (McCulloch et al. 2014; Foster et al. 2013). Given the sensitivity of δ¹¹B⁰CaCO₃-based estimates of pH(calcification site pH) to the analytical uncertainty cited above, two different injection methods (described below) were evaluated to determine what method is most suitable for minimizing analytical error.

2.7.1 Demountable direct injection nebulizer

Memory effects were addressed by introducing samples to the plasma with demountable direct injection high-efficiency nebulizer (d-DIHEN; Louvat et al., 2014). Baseline B-concentrations between samples were measured with counting times of 30 s (Table 2).

2.7.2 Ammonia addition

For the ammonia addition method, a dual inlet PFA Teflon spray chamber was used with an ESI PFA 50 µL/min nebuliser to add ammonia gas at a rate of ~3 mL/min (Al-Ammar et al., 2000; Foster, 2008). The addition of ammonia gas to the spray chamber ensures that the analyte remains alkaline, which prevents volatile boron from recondensing in the chamber during analysis (Al-Ammar et al., 2000). The measured B isotope signal of the rinse blank was then subtracted from the B isotope ratios in order to monitor B wash out, as suggested by Foster (2008). In all cases, wash out time was 200 seconds and samples were matrix- and intensity-matched to the bracketing standards.

3 Results

3.1 Method development

3.1.1 Yield and reproducibility

The yields for boron extraction for both methods were evaluated for various biogenic CaCO₃ samples and were typically between 97 and 102 % (determined by HR-ICPMS; see section 2.6). Washes with pH-buffered MQ-H₂O effectively removed Ca (99.9 %), Na (100 %), Ba (> 80 %), and U (> 93 %) from the sample matrix. The robustness of the methods is demonstrated by the observed agreement (represented as 2 standard deviations around the mean; 2SD) between measured values of the
international CaCO₃ standards JCp-1 and JCt-1, a coral (Porites sp.; δ¹¹B_JCP = 24.45 ± 0.28 ‰, δ¹¹B_JCT = 24.30 ± 0.16 ‰) and a giant clam (Tridacna gigas; δ¹¹B = 16.65 ± 0.27 ‰, δ¹¹B_JCHN = 17.5 ± 0.60 ‰), and their values established via inter-laboratory calibration (δ¹¹B = 24.36 ± 0.51 ‰, n = 10 and 16.34 +/- 0.64 ‰, respectively; Gutjahr et al. 2014; see Table 3). In addition, both column and batch methods were evaluated using the NEP laboratory standard (Porites sp.), a temperate urchin, a hard clam, and an oyster. As shown in Table 3, good agreement was achieved between δ¹¹B_CaCO₃ obtained via the batch and column chemistry methods for each of the biogenic CaCO₃ samples analysed.

4.1 Appropriateness of method for analysing δ¹¹B_CaCO₃ in marine CaCO₃ samples

This study describes extensive method development and analytical validation used to establish stable boron isotope measurements at Ifremer (Plouzané, France), including comparisons of different techniques for sample preparation and sample introduction to the mass spectrometer. For each of the samples evaluated, neither cleaning protocol, nor method of sample
preparation, nor injection system was found to cause a significant difference in $\delta^{11}B_{CaCO_3}$ composition of the samples (Table 3). The most effective method for minimizing memory effects in d-DiII|EN analyses was found to be d-DiII|EN (Louvat et al., 2011). However, d-DiII|EN has a complicated set-up and often generates capillary blockages arising from the aspiration of particles (e.g., resin), and/or from plasma extinction resulting from air bubble introduction. In short, sample analysis via d-DiII|EN requires nearly continuous use to maintain its stability. In contrast, the ammonia-addition method (Al-Ammar et al., 1999, 2000) requires continuous attention by personnel while in use, due to the use of ammonia gas, but is set-up and disassembled with relative ease between uses. We found that a constant ammonia flow of 3 mL/min was necessary to maintain a sufficiently high pH to enable a fast rinse. Less than a 3% boron memory effect was stable after 2 minutes, enabling a signal correction for the sample that follows. Both the column and batch method of B separation yielded low blanks when < 60 µL of resin was used (see sections 2.5 and 2.6). However, the batch method was identified as preferable over the column chemistry method since the batch method has a reduced risk of B contamination due to reduced contact time with air and the small volumes of both resin and acids used in the separation process.

4.2 The $\delta^{11}B_{CaCO_3}$ compositions offer a diverse range of marine calcifiers

The six species investigated exhibited a broad spectrum of $\delta^{11}B_{CaCO_3}$ compositions, ranging from 16.03 ‰ to 35.89 ‰ (Table 4). Assuming that only the borate ion is incorporated into CaCO$_3$ structures, the wide variation in $\delta^{11}B_{CaCO_3}$ (ca. 20 ‰) amongst the investigated species, reared under equivalent thermo-chemical conditions, may arise from inherent differences in pH$_{calcification}$ amongst the species. If this is the case, then the observed range in $\delta^{11}B_{CaCO_3}$ amongst the species (16.03 ‰ to 35.89 ‰) translates to an approximate range in $\text{pH}_{calcification}$ of 7.9 – 9.4.

Boron co-precipitation with inorganic (i.e., abiogenic) CaCO$_3$ (i.e., abiogenic) is known to be dependent on solution pH and is a diverse range of marine calcifiers. However, the relative abundances of the inorganic B species in solution that are incorporated into inorganic CaCO$_3$ (borate ion and boric acid) have been shown to be independent of the parent solution pH (Mavromatis et al. 2015). Although Mavromatis et al. (2015) also found that polymorph mineralogy influences both the B/Ca ratio (higher in aragonite than calcite) and speciation of B in inorganic CaCO$_3$ (borate/boric acid ratio higher in aragonite than in calcite), polymorph mineralogy was not found to influence boron isotope fractionation. B incorporation alone does not appear to influence boron isotope fractionation (Noireaux et al. 2015). Furthermore, because the borate/boric acid ratio is higher in aragonite than in calcite, aragonite-producing species (urchins, coralline algae, oysters) if shell mineralogy was the primary driver of the observed interspecific variation in $\delta^{11}B_{CaCO_3}$ compositions – a trend that is not observed (Fig. 5). Thus, interspecific differences in polymorph mineralogy cannot, alone, explain the species’ disparate $\delta^{11}B_{CaCO_3}$ compositions. The more parsimonious explanation for these observed differences in $\delta^{11}B_{CaCO_3}$ appears to be differences pH$_{calcification}$ in pH at the site of calcification, which would change the speciation of dissolved B at the site of calcification, and therefore the isotopic composition of the borate ion that is preferentially incorporated into the organisms’ CaCO$_3$.

Significant deviations from equilibrium exist in the stable isotopes compositions (e.g., O, C, B) of biogenic marine CaCO$_3$ (e.g., Hemming and Hanson, 1992; McConnaughey, 1989). Notably, many marine calcifiers have $\delta^{11}B$ values that differ from the $\delta^{11}B$ composition of borate ions dissolved in seawater at an equivalent pH (Figs. 3 and 5). When interpreted in the context of the framework that skeletal $\delta^{11}B$ reflects pH$_{calcification}$ rather than pH$_{sea}$ of the organism’s surrounding seawater, these results suggest that marine calcifiers are precipitating their CaCO$_3$ from a discrete fluid with a pH$_{sea}$ higher than, equal to, or, for some species, below that of seawater. A second hypothesis is that whilst seawater pH exerts some control over borate $\delta^{11}B$ at the site of calcification and, hence, $\delta^{11}B_{CaCO_3}$, there are other species-specific effects that may influence $\delta^{11}B_{CaCO_3}$.
composition. The compatibility of these two hypotheses with existing models of biomineralization and observed \(\delta^{11}B_{\text{CaCO}_3}\) for the various marine calcifiers investigated in the present study are discussed below.

### 4.2.1 Temperate coral

The average \(\delta^{11}B_{\text{CaCO}_3}\) for the temperate coral *O. arbuscula* evaluated in this study (24.12 ± 0.19 ‰; n = 3; Tables 3 and 4) is similar to consistent with other literature-based values determined previously published values for aragonitic corals (Table 5; see references therein). Generally, aragonitic corals are enriched in \(\delta^{11}B\) when compared with a theoretical borate \(\delta^{11}B\)-pH curve (see Figures 2 and 4 in 3 and 5). The main vital effect typically used to describe \(\delta^{11}B\)-enrichment in corals, relative to seawater, is an increase in \(\text{H}_2\text{OB}^+\text{calcifying fluid}\) pH at the coral’s site of calcification (e.g., Rollion-Bard et al., 2011b; Trotter et al., 2011; Anagnostou et al., 2012; McCulloch et al., 2012; Wall et al., 2016). This hypothesis is supported by in situ measurements of pH using microelectrodes (e.g., Al-Horani et al., 2003; Ries, 2011), boron isotope analyses (e.g., Anagnostou et al., 2012; Krief et al., 2010; Trotter et al., 2011; McCulloch et al., 2012; Wall et al., 2016), and pH-sensitive fluorescent pH-dye data (Venn et al., 2009, 2011, 2013).

### 4.2.2 Coralline red alga

The average \(\delta^{11}B_{\text{CaCO}_3}\) for the branching, nonarticulated coralline red algal *Neogoniolithon* sp. evaluated in this study (35.89 ± 3.71 ‰; n = 3; Tables 3 and 4) is higher than the \(\delta^{11}B_{\text{CaCO}_3}\) composition of any other calcifying marine organism evaluated to date (Table 5). Of particular interest, one of the coralline red algal specimens evaluated in this study exhibited \(\delta^{11}B_{\text{CaCO}_3}\) (39.94 ‰, Table 3) similar to the average \(\delta^{11}B_{\text{coralline algal skeleton}}\) (i.e., comprising the \(\delta^{11}B\) composition of both dissolved borate and boronic acid; 39.61 ‰) determined by Foster et al. (2010), raising the possibility that coralline red alga incorporate both species of dissolved inorganic boron during calcification. In support of this argument, Cusack et al. (2015) provide NMR data indicating that 30% of the B incorporated into the coralline red alga *Lithothamnion glaciale* was present as boric acid.

However, since the coralline red algae were reared at a pH of 8.1, the \(\delta^{11}B_{\text{CaCO}_3}\) compositions observed for the coralline algae in the present study would require incorporation of both inorganic species of boron at [B(OH)\(_3\)]/[B(OH)\(_2\)] ratios of ca. 75:25, which contradicts not consistent with prior the observations for inorganic and/or organic calcite (e.g., Cusack et al., 2015) reported estimated 30% trigonal B in the calcite lattice of a different species of coralline algae. Therefore, boric acid incorporation cannot alone rule out \(\text{pH}_{\text{CF}}\) as a potential driver of the anomalously elevated \(\delta^{11}B_{\text{CaCO}_3}\) observed here for coralline algae. We can not rule out the potential of pH up-regulation in this species not contradict is not consistent with observed \(\delta^{11}B_{\text{CaCO}_3}\) compositions observed for the coralline red algae. Hence, \(\delta^{11}B_{\text{CaCO}_3}\) compositions of coralline algae data may indeed reflect substantially elevated \(\text{pH}_{\text{CF}}\) at the site of calcification (9.4; Table 4, Fig. 4), suggesting that coralline red algae are highly efficient at removing protons and/or dissolved inorganic carbon from their calcifying medium. Yet another explanation is that \(\delta^{11}B_{\text{CaCO}_3}\) of coralline algal is reflecting elevated pH at the algae’s seawater boundary layer, driven by the alga’s photosynthetic drawdown of aqueous CO\(_2\).
4.2.3 Tropical and temperate urchins

The average $\delta^{11}B_{\text{CaCO}_3}$ for the tropical urchin *E. trubuloids* (18.71 ± 0.26 ‰; n = 3; Tables 3 and 4) and the temperate urchin (*A. punctulata*; -16.28 ± 0.86 ‰; n = 3; Tables 3 and 4), both evaluated in this study and reared at equivalent seawater conditions ($pH_{\text{sw}} = 8.0; 25 ^\circ$C; 32 psu; Table 4), are less than $\delta^{11}B_{\text{CaCO}_3}$ previously reported for other echinoid species (see Table 4; 22.7 ‰ - 22.8 ‰) but are close to theoretical values of dissolved borate at those seawater conditions (17.33 ‰; Fig. 4). Micro-electrode evidence suggests that urchins calciﬁe using from fluids with a pH$_{\text{CF}}$ and composition similar to that of seawater (Stumpp et al. 2012), which is supported by our observation that urchin $\delta^{11}B_{\text{CaCO}_3}$ is similar to $\delta^{11}B$ of dissolved borate. The difference between the $\delta^{11}B_{\text{CaCO}_3}$ of these two species of urchin and the theoretical value of $\delta^{11}B$ for seawater borate calculated $\delta^{11}B_{\text{CaCO}_3}$ (17.33 ‰) is +1.38 ‰ for the tropical urchin and -1.05 ‰ for the temperate urchin—a difference that exceeds their interspecimen variability (± 0.26 ‰ for the tropical urchin; ± 0.86 ‰ for the temperate urchin, determined as standard deviation (SD), see Table 5). However, the urchins could achieve this deviation in $\delta^{11}B_{\text{CaCO}_3}$ by adjusting pH of their calciﬁcing environment by only ± 0.1 units (e.g., pH$_{\text{CF}}$ calciﬁcation-site pH of 8.1 and 7.9 yield $\delta^{11}B$ of calcification site borate of 18.38% and 16.42%, respectively; see Table 4). Thus, if deviations in urchin $\delta^{11}B_{\text{CaCO}_3}$ from seawater borate $\delta^{11}B$ indeed reﬂect urchins’ ability to modify pH at their site of calciﬁcation, these modiﬁcations appear to be relatively minor (i.e., ± 0.1 pH units) and not always in a direction that favours calciﬁcation—consistent with Stumpp et al.’s (2012) observation that urchin biomineralization can occur in cellular compartments where pH$_{\text{CF}}$ is lower than that of seawater. **Note that the $\delta^{11}B$ values for these high-Mg calcite-precipitating organisms are also not consistent with significant boric acid incorporation into the carbonate lattice (Fig. 5).**

4.2.4 Serpulid worm tube

The average $\delta^{11}B_{\text{CaCO}_3}$ for the calcareous tube of the serpulid worm *H. crucigera* evaluated in this study (19.26 ± 0.16 ‰; n = 3; Tables 3 and 4) is close to theoretical values of $\delta^{11}B$ for seawater borate (Fig. 4). The serpulid worm secretes calcareous tubes with *H. crucigera* being the only species that secretes their calcareous tube from a combination of aragonite and high-Mg calcite (HMC; Ries, 2011b). In order to produce their calcareous tubes, *H. crucigera* The worms initially produces a slurry of CaCO$_3$ granules in a pair of anterior glands, which ultimately coalesces within a matrix of inorganic and organic components (Hadley, 1956). The samples of *H. crucigera* evaluated in this study were exposed to environmental conditions ($pH_{\text{sw}} = 8.1; 25 ^\circ$C; 32 psu; Table 4) yielding a theoretical seawater borate $\delta^{11}B$ and, thus, $\delta^{11}B_{\text{CaCO}_3}$ of 18.38 ‰, which is 0.88 ‰ less than $\delta^{11}B_{\text{CaCO}_3}$ measured for *H. crucigera*. Similar to the tropical urchin discussed above, the serpulid worm could generate this divergence in $\delta^{11}B_{\text{CaCO}_3}$ from seawater borate $\delta^{11}B$ by elevating pH$_{\text{CF}}$ pH at its site of calciﬁcation by 0.08 units relative to pHsw. It should be noted that by producing their tubes from a mixture of aragonite and HMC, serpulid worm biomineralization and the resulting CaCO$_3$ matrix is fundamentally different than that of the other marine calcifiers evaluated in this study, which are predominantly monomineralic. **Notably, to our knowledge, these are the ﬁrst published reported $\delta^{11}B_{\text{CaCO}_3}$ data B isotope measurements for serpulid worm tubes and the $\delta^{11}B$ values for this mixed mineralogy precipitating organism is not consistent with significant boric acid incorporation into the carbonate lattice (Fig. 5).**

4.2.5 American oyster

The $\delta^{11}B_{\text{CaCO}_3}$ for the American oyster *C. virginica* evaluated in this study (16.03 ‰; n = 1; Tables 3 and 4) is less than the theoretical values of $\delta^{11}B$ for seawater borate (Fig. 4). Mollusks, such as oysters, construct their shells of LMC (aragonite during the larval stage) from a discrete calciﬁying ﬂuid known as the extrapallial fluid (‘EPF’; e.g., Crenshaw, 1972), with
hemocytes and organic templates playing a potentially important role in crystal nucleation (e.g., Wilbur and Saleuddin 1983; Wheeler 1992; Marie et al., 2012; Weiner et al., 1984; Mount et al., 2004). The sample of *C. virginica* evaluated in this study was exposed to seawater conditions \((pH_{EPF} = 8.2; 25 \, ^\circ C; 32 \, psu; \text{Table 4})\) that yield a theoretical borate \(\delta^{11}B\), and thus \(\delta^{11}B_{\text{ACO}_3}\), of 19.57 \%, which is 3.54 \% greater than \(\delta^{11}B_{\text{ACO}_3}\) measured for *C. virginica*. The observation that oyster \(\delta^{11}B_{\text{ACO}_3}\) is substantially less than theoretical \(\delta^{11}B\) of seawater borate suggests that \(pH_{EPF}\) of oyster extrapallial fluid \(pH\) is less than the \(pH\) of the oyster’s surrounding seawater. Indeed, \(pH\) microelectrode measurements show that \(pH\) of oyster EPF \((pH_{EPF})\) is approximately 0.5 units less than seawater \(pH\), which the author attributes to metabolically driven accumulation of dissolved CO\(_2\) when the oyster’s shell is closed (Crenshaw, 1972; Littlewood and Young, 1994; Michaelidis et al., 2005). Oysters appear to overcome low CaCO\(_3\) saturation state in the EPF, compared to corals that maintain elevated CaCO\(_3\) saturation state at their site of calcification, by using organic templates to facilitate biomineral growth (e.g., Marie et al., 2012; Addadi et al., 2003; Weiner et al., 1984). The oyster could generate this negative divergence in \(\delta^{11}B_{\text{ACO}_3}\) from seawater borate \(\delta^{11}B\) by decreasing \(pH_{EPF}\) \(pH\) at the site of calcification by 0.35 units \((\text{Table 4})\), which, given the proximity of the independent \(pH\)-microelectrode measurements of oyster EPF, seems to be a plausible explanation for why oyster \(\delta^{11}B_{\text{ACO}_3}\) falls below the theoretical borate \(\delta^{11}B\)-\(pH\) curve \((\text{Klochko et al., 2009; Fig 5})\). To our knowledge, these are the first reported \(B\) isotope measurements for Notably, these are the first published \(\delta^{11}B_{\text{ACO}_3}\) data for oysters and the \(\delta^{11}B\) values for this low Mg calcite-precipitating organism are not consistent with significant boric acid incorporation into the carbonate lattice \((\text{Fig. 5})\).

4.3 What does a species’ \(\delta^{11}B_{\text{ACO}_3}\) reveal about its calcification site \(pH\) and relative sensitivity to ocean acidification?

Understanding how marine organisms calcify is a critical requirement for understanding and, ideally, predicting their physiological response to future ocean acidification (e.g., Kloppas et al., 2006). Given that all 6 species were grown under nearly equivalent controlled laboratory conditions, the observed interspecific range in \(\delta^{11}B_{\text{ACO}_3}\) supports the hypothesis that B-isotope fractionation in marine calcifiers is species dependent \((\text{Table 5 and references therein})\). We hypothesize that this species-dependent variability is driven by interspecific differences in \(pH\) at the site of calcification. To explore this hypothesis, \(\delta^{11}B_{\text{ACO}_3}\) values were converted to calcification site \(pH\) \((pH_{CS})\) from measured seawater temperature, salinity, seawater \(\delta^{11}B\) value of 39.61 ± 0.04 ‰ \((\text{Foster et al., 2010})\), and a B-isotope fractionation factor of 1.0272 \((\text{Klochko et al., 2006, Table 4})\). These calculations yield a calcification site \(pH\) \((\text{Table 4})\) of 8.5 for the temperate coral \((O. arbuscula), 8.4\) for the coralline red alga \((N. virgatilis)\), \(8.1\) for the tropical urchin \((E. tribuloides), 7.9\) for the temperate urchin \((H. crucigera), 7.9\) for the American oyster \((C. virginica). NOTABLY, the temperature coral \((O. arbuscula)\) and coralline red alga \((N. virgatilis)\) have higher calculated calcification site \(pH\) \((\text{based on their boron isotope composition})\) than the other organisms. As discussed above \((\text{section 4.2})\), one possible explanation for these differences is that corals and potentially coralline red algae maintain their calcifying fluids at a higher \(pH\) than the calcifying fluids of other calcifying marine organisms.

Notably, the different species’ \(\delta^{11}B_{\text{ACO}_3}\) and reconstructed calcification site \(pH\) appeared to exhibit a moderate, inverse relationship with their experimentally determined vulnerability to ocean acidification \((\text{Ries et al., 2009})\), i.e., species exhibiting more resilient ‘parabolic’ \((\text{e.g., coralline red alga})\) and ‘threshold’ \((\text{e.g., coral, tropical urchin})\) responses to ocean acidification generally exhibited a higher \(\delta^{11}B_{\text{ACO}_3}\) and, thus, calcification site \(pH\) than species exhibiting the more vulnerable ‘negative’ responses \((\text{e.g., oyster, serpulid worm})\) to ocean acidification \((\text{Table 4})\). The temperate urchin was the exception to this general trend, as it exhibited a relatively resilient parabolic response to ocean acidification yet maintained \(\delta^{11}B_{\text{ACO}_3}\) and, thus, calcification site \(pH\) close to that of seawater.
In the absence of empirical measurements of calcifying fluid temperature, salinity, and \( \delta^{11}B \), these parameters are generally assumed to reflect seawater. However, the large variability in the calculated calcification site pH for these organisms (e.g., Tables 4 and 5) suggests that biological processes (e.g., regulation of calcification site pH) are governing boron isotope fractionation within the calcifying fluids and shells of marine calcifiers. Below, we evaluate the sensitivity of calculating calcification site pH from measured \( \delta^{11}B_{\text{CaCO}_3} \) composition by testing the factors that may influence the theoretical model of borate \( \delta^{11}B \) variation as a function of pH; namely, \( pK_{\alpha} \) and \( \alpha \).

4.3.1 Sensitivity of \( \delta^{11}B_{\text{CaCO}_3} \) composition to choice of \( pK_{\alpha} \) and \( \alpha \)

Here, we evaluate the sensitivity of calculating calcification site pH from measured \( \delta^{11}B_{\text{CaCO}_3} \) composition by testing the two principal factors that may influence the theoretical model of borate \( \delta^{11}B \) variation as a function of both seawater pH and calcification site pH; namely, \( pK_{\alpha} \) and \( \alpha \). Other factors, such as the modification of seawater pH and incorporation of boric acid by marine calcifiers, are not presented here as they are discussed previously (see Section 4.2, Fig. 5). Notably, the temperature coral (H. crucigera) and coralline red alga (Neogoniolithion sp.) have higher calculated pH values (based on their boron isotope composition) than the other organisms.

4.3.3 Sensitivity analysis of \( \delta^{11}B_{\text{CaCO}_3} \)-derived calculations of pH\(_{\text{SEW}} \) to choice of \( pK_{\alpha} \) and \( \alpha \)

Here, we evaluate the sensitivity of calculating seawater pH from measured \( \delta^{11}B_{\text{CaCO}_3} \) composition by testing the factors that may influence the theoretical model of borate \( \delta^{11}B \) variation as a function of pH; namely, \( pK_{\alpha} \) and \( \alpha \). A sensitivity analysis of \( \delta^{11}B \) in seawater was not conducted since all organisms evaluated in this study were exposed to seawater from the same source and, thus, of identical \( \delta^{11}B \) composition.

4.3.4.3.1 Sensitivity analysis of \( \delta^{11}B \)-derived pH\(_{\text{SEW}} \) to \( pK_{\alpha} \)

Sensitivity of \( \delta^{11}B_{\text{CaCO}_3} \)-derived pH\(_{\text{SEW}} \).
The determination of pH$_{CF}$ from $pK_b$ and the $\delta^{11}B$ of calcifying fluid (δ$^{11}B_{CF}$) of seawater and δ$^{11}B_{CaCO_3}$ (δ$^{11}B_{B(OH)4}$)
 can be summarized with the following equation (Eq. 1):

\[
pH_{CF} = pK_b - \log ((\delta^{11}B_{B(OH)4}) + \delta^{11}B_{CaCO_3}) - (\alpha \times \delta^{11}B_{B(OH)4}) - 1000(\alpha - 1));
\]

where $pK_b$ is 8.6152 (at 25°C and 32 psu; Dickson, 1990), δ$^{11}B_{B(OH)4}$ is 39.61 ‰ (inherited from δ$^{11}B_{SW}$ Foster et al., 2010), and $\alpha$ is 1.0272 (Klochko et al., 2006).

Thus, δ$^{11}B_{B(OH)4}$-δ$^{11}B_{CaCO_3}$ can be calculated across a range of pH$_{Bk}$-pH$_{CF}$ (Fig. 1b; Table S1).

It is important to note that the difference in δ$^{11}B_{CaCO_3}$ between each pH unit (when fluid pH < $pK_a$) increases with pH, as shown in Fig. 1b (see also Table S1). For example, a change in pH from 7.75 to 7.80 predicts a δ$^{11}B_{CaCO_3}$ difference of 0.35 ‰ (15.77 ‰ - 15.42 ‰), whereas a change in pH from 8.35 to 8.40 predicts a δ$^{11}B_{CaCO_3}$ difference of 0.74 ‰ (22.59 ‰ - 21.85 ‰). Thus, the relationship between fluid-pH$_{CF}$ and δ$^{11}B_{CaCO_3}$ is nonlinear over the range of fluid-pH$_{CF}$ of interest (7 < pH < 10), with pH having the greatest influence on δ$^{11}B_{CaCO_3}$ as fluid pH$_{CF}$ approaches pK$_b$.

As discussed above (section 4.2), most marine calcifiers are thought to precipitate CaCO$_3$ from a discrete ‘calcifying fluid’, which appears to be derived, yet physically separated, from seawater and has with a pH greater than (e.g., coralline alga, corals), equivalent to (e.g., serpulid worm, urchins), or less than (e.g., oysters) seawater. Although the sensitivity analysis for the δ$^{11}B_{CaCO_3}$-derived determinations of pH$_{CF}$ at a $pK_b$ of 8.6152 indicates that a small change in pH$_{CF}$ calcification site pH will greatly influences δ$^{11}B_{CaCO_3}$, especially as pH approaches pK$_b$ (8.6152), the range of the organisms’ seawater pH (8.0-8.2; Table 4) could only account for a theoretical 2.24 ‰ range in δ$^{11}B_{CaCO_3}$ (Table S1), far less than the ca. 20 ‰ range that was observed. It therefore follows that the large variability in δ$^{11}B_{CaCO_3}$ (ca. 20 ‰) observed for the investigated species requires an alternative explanation, such as changes in pH$_{CF}$ calcification site pH—particularly for the coralline alga, coral and oyster species that exhibited such large deviations in predicted vs. observed δ$^{11}B_{CaCO_3}$ (see section 4.2).

### 4.3.2 Sensitivity analysis of δ$^{11}B$-derived pH$_{CF}$ to choice of $\alpha$.

As discussed in the Introduction (section 1.1), much work has gone into establishing a pH-dependent relationship between the δ$^{11}B$ of dissolved borate and boric acid in seawater, and pH (see Xiao et al., 2014; for detailed discussion), with the earliest published palaeo-pH reconstructions using a theoretical value of 1.0194 (Kakihana et al., 1977; see Fig. 32). An empirical α of 1.0272 (Klochko et al., 2006) has now been shown to better predict borate δ$^{11}B_{B(OH)4}$, viz. δ$^{11}B_{CaCO_3}$, across a range of pH relevant for seawater (Rollion-Bard and Erer, 2010; Xiao et al., 2014). However, δ$^{11}B_{CaCO_3}$ of many species of calcifying marine organisms fall either above or below the theoretical borate δ$^{11}B_{B(OH)4}$-pH curves. It has long been suggested (and shown for corals) that calcifying organisms diverge from the predicted δ$^{11}B_{CaCO_3}$ due to their ability to modify pH of their calcifying environments (e.g., Anagnostou et al., 2012; Hönisch et al., 2004; Krief et al., 2010; Rae et al., 2011; Reynaud et al., 2004; Trotter et al., 2011; McCulloch et al., 2012; Wall et al., 2016). In the present study, species-specific divergences in δ$^{11}B_{CaCO_3}$ from the theoretical δ$^{11}B_{B(OH)4}$-pH borate δ$^{11}B$-pH-curves are interpreted as evidence of the differing capacities of calcifying marine species to modified pH$_{CF}$ at the organism’s site of calcification. Importantly, existing models of biominalization for each species are generally compatible with these δ$^{11}B_{CaCO_3}$-derived estimates of pH$_{CF}$ calcification site pH (see section 4.2).
Although an $\alpha$ of 1.0272 (Klochko et al., 2006) was used in the present study to estimate $\text{calcification site pH}_{\text{CF}}$, other theoretical values for $\alpha$, yielding slightly different borate $\delta^{11}B$-pH curves (e.g., Byrne et al., 2006; Palmer et al., 1987; see Fig. 4), will of course yield slightly different estimates of $\text{pH}_{\text{CF}}$ at the calcification sites of each organism. For example, using $\alpha$ values of 1.033 (Palmer et al., 1987), 1.0285 (Byrne et al., 2006), 1.0272 (Klochko et al. 2006), and 1.0194 (Kakihana et al. 1977) and a $\delta^{11}B_{\text{B_CaCO}_3}$ of 24.12‰ (temperate coral; $\text{pH}_{\text{CF}} = 8.1$) yields $\text{pH}_{\text{CF}}$ of $8.7, 8.6, 8.5, \text{and} 8.1$, respectively—a difference of 0.6 pH units. It should also be noted that the lower the $\delta^{11}B_{\text{B_CaCO}_3}$ the more sensitive the reconstructed pH is to choice of $\alpha$. For example, changing $\alpha$ from 1.0272 to 1.0330 will result in a 0.24 pH unit shift for $\delta^{11}B_{\text{B_CaCO}_3} = 20$‰, but only a 0.12 and 0.08 pH unit shift for $\delta^{11}B_{\text{B_CaCO}_3} = 30$‰ and 39.5‰, respectively. This underscores the importance of using the same $\alpha$ when comparing $\text{pH}_{\text{CF}}$ amongst species.

### 4.4.3.3 Implications of $\delta^{11}B_{\text{B_CaCO}_3}$-derived estimates of $\text{calcification site pH}_{\text{CF}}$ for species-specific vulnerability to ocean acidification

Regardless of which $\alpha$ value is used, a wide range (ca. 20‰) of $\delta^{11}B_{\text{B_CaCO}_3}$ compositions is observed amongst the marine calcifying species investigated. Furthermore, there appears to be a moderate inverse relationship between the species’ relative ability to elevate $\text{pH}_{\text{CF}}$, calcification site pH, and their empirically determined vulnerability to ocean acidification (see Ries et al., 2009 and Table 4). These results support the assertion that interspecific differences in $\text{pH}_{\text{CF}}$ at the calcification site pH contribute to marine calcifiers’ differential responses to ocean acidification—highlighting the need for future queries into the mechanisms driving boron isotope fractionation and biomineralization within marine calcifying organisms.

Understanding how marine organisms calcify is a critical requirement for understanding and, ideally, predicting their physiological responses to future ocean acidification (e.g., Kleypas et al., 2006). Notably, the different species’ $\delta^{11}B_{\text{B_CaCO}_3}$ and reconstructed $\text{pH}_{\text{CF}}$ appeared to exhibit a moderate, inverse relationship with their experimentally determined vulnerability to ocean acidification (Ries et al., 2009). Specifically, species exhibiting more resilient ‘parabolic’ (e.g., coralline red alga) and ‘threshold’ (e.g., coral, tropical urchin) responses to ocean acidification generally exhibited a higher $\delta^{11}B_{\text{B_CaCO}_3}$ and, thus, $\text{pH}_{\text{CF}}$ than species exhibiting the more vulnerable ‘negative’ responses (e.g., oyster, serpulid worm) to ocean acidification (Table 4). The temperate urchin was the exception to this general trend, as it exhibited a relatively resilient parabolic response to ocean acidification yet maintained $\delta^{11}B_{\text{B_CaCO}_3}$ and, thus, $\text{pH}_{\text{CF}}$ close to that of $\text{pH}_{\text{SW}}$. These results support the assertion that interspecific differences in $\text{pH}_{\text{CF}}$ at the calcification site pH contribute to marine calcifiers’ differential responses to ocean acidification—highlighting the need for future queries into the mechanisms driving boron isotope fractionation and biomineralization within marine calcifying organisms.

Given that all 6 species were grown under nearly equivalent controlled laboratory conditions, the observed interspecific range in $\delta^{11}B_{\text{B_CaCO}_3}$ supports the hypothesis that boron isotope fractionation in marine calcifiers cannot be explained solely by borate incorporation at ambient $\text{pH}_{\text{SW}}$ (see Table 5 and references therein). We hypothesize that this species-dependent variability is driven by interspecific differences in $\text{pH}_{\text{SW}}$. To explore this hypothesis, $\delta^{11}B_{\text{B_CaCO}_3}$ values were converted to $\text{pH}_{\text{SW}}$ from measured seawater temperature, salinity, seawater $\delta^{11}B$-value of 20.61±0.20‰ (Foster et al., 2010), and an $\alpha$ of 1.0272 (Klochko et al., 2006; Table 4). These calculations yield a $\text{pH}_{\text{SW}}$—assuming that only borate is incorporated—of 8.5 for the temperate coral (O. arbuscula), 9.4 for the coralline red alga (Neogoniolithion sp.), 8.1 for the tropical urchin (*H. crucigera*), 7.9 for the temperate urchin (*A. punctulata*), 8.2 for the serpulid worm (*H. crassipenis*), and 7.9 for the American oyster (*C. virginica*). Notably, the temperature coral (O. arbuscula) and coralline red algae (Neogoniolithion sp.) have higher calculated $\text{pH}_{\text{SW}}$ (based on their boron isotope composition) than the other organisms. As discussed above.
(section 4.2), one possible explanation for these differences is that corals (and potentially coralline red algae) maintain their calcifying fluids at a higher pH than the calcifying fluids of other calcifying marine organisms.

Notably, the different species’ $\delta^{11}B_{\text{CaCO}_3}$ and reconstructed pH$_{\text{cf}}$ appeared to exhibit a moderate, inverse relationship with their experimentally determined vulnerability to ocean acidification (Ries et al., 2000)—i.e., species exhibiting more resilient ‘parabolic’ (e.g. coralline red alga) and ‘threshold’ (e.g. coral, tropical urchin) responses to ocean acidification generally exhibited a higher $\delta^{11}B_{\text{CaCO}_3}$ and, thus, pH$_{\text{cf}}$, than species exhibiting the more vulnerable ‘negative’ responses (e.g. oyster, serpulid worm) to ocean acidification (Table 4). The temperate urchin was the exception to this general trend, as it exhibited a relatively resilient parabolic response to ocean acidification yet maintained $\delta^{11}B_{\text{CaCO}_3}$ and, thus, pH$_{\text{cf}}$ close to that of pH$_{\text{sw}}$.

In the absence of empirical measurements of calcifying fluid temperature, salinity, and $\delta^{11}B$; these parameters are generally assumed to reflect seawater. However, the large variability in the calculated pH$_{\text{cf}}$ for these organisms (e.g. 7.9-9.4; Table 4) that were grown in near identical seawater conditions (pH$_{\text{sw}}$: 8.0-8.2; Table 4) suggests that a biological process (e.g. regulation of pH$_{\text{cf}}$) is governing boron isotope fractionation within the calcifying fluids and shells of marine calcifiers.

4.3.4 Further calibration of the $\delta^{11}B_{\text{CaCO}_3}$-derived determinations of pH

The observed deviations of the investigated species’ $\delta^{11}B_{\text{CaCO}_3}$ from the borate $\delta^{11}B$-pH curve also highlight that, in some species, paleo-seawater pH may not simply be reconstructed by projecting measured $\delta^{11}B_{\text{CaCO}_3}$ onto a theoretical seawater $\delta^{11}B_{\text{borate}}$-borate $\delta^{11}B$-pH curve (see also Sanyal et al., 1996; Sanyal et al., 2001; Honisch et al., 2003; Trotter et al., 2011; Anagnostou et al., 2012). Instead, the model species used for paleo-seawater pH reconstructions may require calibration through controlled laboratory experiments and/or core-top calibrations that empirically define the species-specific relationship between seawater pH and $\delta^{11}B_{\text{CaCO}_3}$.

5 Conclusion

This study establishes the methodology for measuring stable boron isotopes at Ifremer (Plouzané, France) and reveals that neither cleaning protocol (oxidized vs. untreated), nor method of sample preparation (batch vs. column), nor injection system (d-DHIFEN vs. ammonia addition) causes a significant difference in $\delta^{11}B_{\text{CaCO}_3}$ composition of the samples. The batch method of boron extraction is preferred over the column chemistry method since the risk of B contamination is reduced in the batch method due to shorter exposure to potential contaminants and smaller reagent volumes.

This newly established method for measuring stable boron isotopes at Ifremer was used to measure the $\delta^{11}B_{\text{CaCO}_3}$ composition of six species of marine calcifiers that were all grown under equivalent seawater conditions. –The coralline red alga Neogoniolithon sp. (35.89 ± 3.71 ‰; n = 3) exhibited the highest $\delta^{11}B_{\text{CaCO}_3}$, followed by the temperate coral O. arbuscula (24.12 ± 0.19 ‰; n = 3), the tube of the serpulid worm H. crucigera (19.26 ± 0.16 ‰; n = 3), the tropical urchin E. tribuloides (18.71 ± 0.26 ‰; n = 3), the temperate urchin A. punctulata (16.28 ± 0.86 ‰; n = 3), and the American oyster C. virginica (16.03 ± 0.03 ‰; n = 1). The observed ca. 20 ‰ range in $\delta^{11}B_{\text{CaCO}_3}$ composition of the investigated species constitutes the largest range in biogenic $\delta^{11}B_{\text{CaCO}_3}$ reported to date.

Consideration of these extreme interspecific differences in $\delta^{11}B_{\text{CaCO}_3}$ in the context of existing models of biomineralization for the investigated species, combined with published measurements of pH$_{\text{cf}}$-calcification site pH for some of the species, generally supports the assertion that most marine calcifiers precipitate their CaCO$_3$ from a discrete calcifying medium with a pH that is either greater than, equivalent to, or, for some species, less than external seawater pH. Furthermore, the observation
that the different species’ $\delta^{18}$B$_{\text{c}}$CO$_3$ and reconstructed pH$_{\text{calcification site}}$ generally varied inversely with their experimentally determined vulnerability to ocean acidification suggests that a species’ relative resilience (or vulnerability) to OA may be influenced by their ability (or lack thereof) to maintain an elevated pH$_{\text{calc.}}$ at their site of calcification. These observations contribute to the growing body of work that uses $\delta^{18}$B$_{\text{c}}$CO$_3$ as a tool to advance understanding of the mechanisms by which marine calcifiers build and maintain their shells and skeletons and, ultimately, how they will respond to anthropogenic ocean acidification.
References


Cohen, A. L. and McConnaughey, T. A.: Geochemical Perspectives on Coral Mineralization, in Biominerlization, edited by


Tables

Table 1. Protocol used to evaluate the column chemistry method of boron extraction. Three volumes of resin (60, 250 and 500 µL) were evaluated.

<table>
<thead>
<tr>
<th>Step</th>
<th>mg resin</th>
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<th>125</th>
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<td>1</td>
<td>Resin (µL)</td>
<td>60</td>
<td>250</td>
<td>500</td>
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<tr>
<td>2</td>
<td>MQ H2O at pH 7 (mL)</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>3</td>
<td>0.5 N HNO3 (mL)</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>MQ H2O at pH 7 (mL) x3</td>
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<td>2.5</td>
<td>5</td>
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<td>6</td>
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<td>536</td>
<td>536</td>
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<td>2</td>
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Table 2. Mass spectrometer operating conditions.

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<th>d-DHEN</th>
<th>Ammonia Addition</th>
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<tr>
<td><strong>Injection system</strong></td>
<td>Demountable Direct Injection High-efficiency Nebulizer</td>
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<td><strong>Sample Gas Flow Rate</strong></td>
<td>0.3 L min⁻¹, total B</td>
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<td><strong>Running Concentrations</strong></td>
<td>B = 50 ppb</td>
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<td><strong>Sensitivity</strong></td>
<td>35 V ppm⁻¹, total B</td>
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<td><strong>Blank Level</strong></td>
<td>&lt; 0.5 % of ¹¹B signal after 30s in 2 % HNO₃, 0.1 % after 120s</td>
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<td><strong>Resolution</strong></td>
<td>Low</td>
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<td><strong>Forward Power</strong></td>
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<tr>
<td><strong>Accelerating Voltage</strong></td>
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<td><strong>Plasma Mode</strong></td>
<td>Wet Plasma</td>
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<td><strong>Cool Gas Flow Rate</strong></td>
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<td><strong>Auxiliary Gas Flow Rate</strong></td>
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<td><strong>Sampler Cone</strong></td>
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<tr>
<td><strong>Skimmer Cone</strong></td>
<td>X Ni cone</td>
</tr>
<tr>
<td><strong>Interferences</strong></td>
<td>⁴⁰Ar⁺⁺⁺⁺ ²⁰Ne⁺⁺⁺⁺ resolved</td>
</tr>
<tr>
<td><strong>Accuracy</strong></td>
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<tr>
<td><strong>Acquisition</strong></td>
<td>30 x 4s</td>
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<tr>
<td><strong>Baselines</strong></td>
<td>Counting times of 20 s</td>
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Table 3. Boron isotope composition ($\delta^{11}$B; ‰) of all species evaluated, including international carbonate standards JC-1 (Porites sp.) and JCt-1 (Hard clam). Data are presented as average of $n$ analyses and the precision is reported as 2 standard deviations (2SD). The cleaning protocol (Oxidised – ‘Ox’/Uncleaned – ‘U’), separation method (‘column’/‘batch’), and injection method (‘NH$_3$’/‘d-DIHEN’) are presented for comparison.

<table>
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<tr>
<th>Sample type</th>
<th>Name</th>
<th>$\delta^{11}$B (2SD)</th>
<th>$n$</th>
<th>Cleaning</th>
<th>Separation</th>
<th>Injection</th>
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<td>JCt-1</td>
<td>17.50 0.60 12 Ox</td>
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<td>batch</td>
<td>d-DIHEN</td>
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<td>JCt-1</td>
<td>16.90 0.30 6 Ox</td>
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Table 4. Summary of the average and standard deviation (SD) of $\delta^{11}B$ for each species ($\%$), calculated pH at calcification site of calcifying fluid (pH$_{CFS}$), pH of the seawater (pH$_{SW}$) during the experimental conditions, and difference between pH$_{CFS}$ and pH$_{SW}$ ($\Delta$pH). The calcification response to ocean acidification experiments (OA Response); Ries et al., 2009, and shell/skeletal mineralogy (HMC = high-Mg calcite; LMC = low-Mg calcite) were previously described by Ries et al. (Ries et al., 2009). The abbreviations HMC and LMC describe high-Mg calcite and low-Mg calcite. Typically, in most cases 3 biological replicates of each species were measured. ‘NA’ = not available, only one biological replicate analysed.

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Figures

Fig. 1. (a) Speciation of dissolved inorganic boron (B(OH)$_3$ and B(OH)$_4^-$) as a function of seawater pH. (b) δ$^{11}$B of dissolved inorganic boron species as a function of seawater pH. The pKb is 8.6 at 25 °C and 35 psu (Dickson, 1990), α is 1.0272 (Klochko et al., 2006), and δ$^{11}$B$_{W}$ is 39.61 (Foster et al., 2010).

Fig. 2. Plot of literature-derived δ$^{11}$B for corals, foraminifera and brachiopods. The two gray lines indicate the theoretical seawater borate δ$^{11}$B-pH curves that have been applied most frequently to interpret δ$^{11}$B variability in marine calcifiers. The pKb is 8.6152 at 25 °C and 32 psu (Dickson, 1990).

Fig. 3. Elution curves indicating cumulative yield of boron for different volumes of the boron-specific resin (Amberlite IRA 743) placed in an ion exchange column.

Fig. 4. Boron isotopic composition (± SD) of different marine calcifying organisms with respect to seawater pH (± SD). The six species shown in this figure were grown under controlled pCO$_2$ conditions of ca. 409 ppm. Gray lines are theoretical seawater borate δ$^{11}$B$_{B(OH)4^-}$-pH curves based on different fractionation factors (α) that have been used to describe boron isotope fractionation between borate ion and boric acid in seawater (using pKb of 8.6152 at 25°C and 32 psu). Although α = 1.0272 (Klochko et al., 2006) is presently the most commonly used, δ$^{11}$B$_{B(OH)4^-}$-pH curves calculated from other values of α are also shown for reference.

Fig. 5. Exploring the potential influence of pH and boron speciation on carbonate. The influence of pH on the speciation of boron species and δ$^{11}$B (adapted from Rollion-Bard, 2011b). The solid and dashed curves represent the δ$^{11}$B composition that would result from the incorporation of different amounts of B(OH)$_3$ into the marine biogenic carbonates. The dashed vertical lines represent the calculated pH based on the assumption that 0% B(OH)$_3$ is incorporated into the temperate coral skeleton and 0%, 30%, and 75% B(OH)$_3$ is incorporated into the coralline algal skeleton. Of the calcite species examined, only the coralline algae has a δ$^{11}$B composition that could conceivably originate at least in part from B(OH)$_3$ incorporation.
Author Contribution

RAE conceived of the project. RAE and JBR conceived of the project and wrote the proposals that funded the work. JBR cultured the organisms. RAE, JNS, and JBR contributed to experimental design. JNS, Y-WL, MG, EP, and RAE contributed to method development. JNS performed the measurements with assistance from EP. JNS conducted the data analysis. Interpretation was led by JNS and RAE with input from JBR and Y-WL. JNS drafted the paper, which was edited by all authors.
Acknowledgements

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