We would again like 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. Note that the line numbers we refer to in our response to reviewer are from the original resubmitted manuscript (bold) or from the revised manuscript that contains the tracked changes.

Check table #s; they do not correspond to their order in the text.

We have checked over the table numbers and their order in the text. As a result, we have removed one reference to Table 3 on line 248. Here, we changed the text to indicate that there were at least 3 specimens per species evaluated and therefore, the use of the table reference was no longer needed.

L44-48*: I think the presentation would be clearer if this section is moved down to the paragraph starting on line 76. At that point you have introduced the relevant factors (α, pKB*) in lines 49-75.*

We have made the changes recommended by the reviewer.

L71-85 (and including L44-48): *As in the previous version, there is still a misrepresentation of how the community gets between carbonate δ11B measured on a mass spectrometer and pH. You actually get this right at the end of the discussion (L504-509), but this needs to be in the introduction and not the* discussion*. I will revoke any copyright claim to this and recommend the authors include the following:*

First, there is the theoretical model for δ11Bborate varying with pHsw, given by the following equation (please include and cite Zeebe and Wolf-Gladrow, 2001):

$$pH = pK_B^* - \log\left(\frac{\delta^{11}B_{CaCO_3} - \delta^{11}B_{sw}}{\delta^{11}B_{sw} - \alpha_B * \delta^{11}B_{CaCO_3} - \varepsilon_B}\right)$$

Uncertainties in this model stem from uncertainties in the variables pKB, δ11Bsw, and primarily α (as you discussed on L59-71 and evaluate in the discussion). Pagani et al. (2005), Foster and Rae (2016) have nice discussions of these uncertainties that you can cite here.*

2) Using δ11BCaCO3 to derive pHsw requires knowledge of the relationship between δ11BCaCO3 and δ11Bborate. One option is to assume that δ11BCaCO3 =δ11Bborate, as was done by early studies (e.g., Hemming and Hanson, 1992). However, Sanyal et al. (2000-GCA and 2001-Paleoceanography) pointed out that different carbonates (inorganic calcite, O. universa, and G. sacculifer) exhibited offset relationships to δ11Bborate. To quote from the 2001 Paleoceanography paper (emphasis added): "It is noteworthy that empirical δ11B versus pH curves for both biogenic and inorganic calcite plot close to the calculated B(OH)4- curve, indicating that the charged species is preferentially incorporated into the carbonates. A parallel offset, however, was identified between the theoretical B(OH)4-

curve and the empirical δ11B vs. pH curve of both O. universa and inorganic carbonates. This suggests that the calculated B(OH)4- curve cannot be directly applied to estimate paleo-pH from the δ11B of all carbonates."

Thus, it was known that δ11BCaCO3 ≠ δ11Bborate even before Pagani et al. (2005). As a result, empirical calibrations between δ11BCaCO3 and δ11Bborate are needed to calculate pH, as discussed by H.nisch et al. (2007-commment on Pagani et al. 2005), Foster (2008), Henehan et al. (2013), Farmer et al. (2015), Henehan et al. (2016), and Foster and Rae (2016). The current practice of the field is to use these empirical calibrations to calculate δ11Bborate from δ11BCaCO3, and then use the above theoretical model (with its uncertainties) to calculate pHsw. In other words, δ11B-based paleopH reconstructions do not assume that δ11BCaCO3 =δ11Bborate.

This leads in nicely to your Section 1.3 (L138) because your study interrogates one reason why δ11BCaCO3 may not equal δ11Bborate (namely, pHcf ≠ pHsw). In addition, this has one small and one big implication for the manuscript. First, on L71-75 you state "empirical species-specific calibrations between δ11BCaCO3 and pHsw are likely more appropriate than theoretical α values". This is not correct! The empirical species-specific calibrations are presented as between δ11BCaCO3 and δ11Bborate. To get from δ11Bborate to pH requires the theoretical model (equation above), which depends on α. See/cite equation 1 in H.nisch et al. (2007) and equations 24 and 26 in Foster and Rae (2016). To summarize, empirical calibrations are not more appropriate than theoretical α values; in fact they require α, but they are more appropriate than assuming that δ11BCaCO3 =δ11Bborate.

Second, because this manuscript presents δ11BCaCO3 values at only a single pH, you do not know the true relationship between δ11BCaCO3 and δ11Bborate for your calcifiers. Therefore your calculated pH values must assume that δ11BCaCO3 =δ11Bborate. If this study attempted to reconstruct paleo-pH, this assumption would invalidate the reconstruction and the pH values should be treated with suspicion. However, because you do not have a range of pH values for each specimen, you are essentially forced to assume that δ11BCaCO3 =δ11Bborate. That is OK for the purpose of exploratory studies like this one, just be sure to state this assumption explicitly in the text (see my comment on L327). In general, you have done well to highlight this assumption throughout the discussion.

We have made some changes to this section as suggested by the reviewer, although we have not done a copy/paste of the text in the reviewer's comments, as suggested by the reviewer. Alternatively, to clarify our discussion points for this section we have modified some of the text, as the reviewer suggested, to:

(1) include the presentation of the model by Zeebe and Wolf-Gladrow (2001) and to discuss the uncertainties of this model starting at line 100.

(2) we have added the following text to address his second point starting on line 94:

> "paleo-seawater pH may not simply be reconstructed by projecting measured δ^{11}B of calcium carbonate ($\delta^{11}B_{CaCO3}$) onto a theoretical seawater borate δ^{11}B ($\delta^{11}B_{B(OH)4-}$)-pH curve (see also Anagnostou et al., 2012; Honïsch et al., 2003; Sanyal et al., 1996, Sanyal et al., 2001; Trotter et al., 2011). Instead, the 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 (pH_{SW}) and $\delta^{11}B_{CaCO3}$."
>
> And starting on line 107
>
> "Application of this proxy also assumes that $\delta^{11}B_{CaCO3}$ reflects seawater $\delta^{11}B_{B(OH)4-}$ and, thus, seawater pH (Hemming and Hanson, 1992). Although early studies assumed that $\delta^{11}B_{CaCO3}$ was indeed equivalent to seawater $\delta^{11}B_{B(OH)4}^{-}$ (e.g., Hemming and Hanson, 1992), Sanyal et al. (2000, 2001) observed that empirically derived $\delta^{11}B_{CaCO3}$-pH curves of biogenic and abiogenic calcites were parallel but vertically offset from the theoretical $\delta^{11}B_{B(OH)4-}$-pH curve, which led them to conclude that paleo-seawater pH cannot always be directly calculated from $\delta^{11}B_{CaCO3}$ using the theoretical $\delta^{11}B_{B(OH)4-}$-pH relationship (i.e., $\delta^{11}B_{CaCO3}$-pH relationships must be empirically calibrated for the species hosting the paleo-pH proxy).

We hope that the reviewer is satisfied with the changes that we have made.

L108: *remove "forecasted," atmospheric pCO_2 has definitely risen!*

We have made the changes recommended by the reviewer.

L114: *change "that they need" to "needed"*

We have made the changes recommended by the reviewer.

L170-171: *Specify what efforts were made to minimize sample exposure to laboratory air. Did you take the caps off just before analysis?*

We have made the changes recommended by the reviewer. The sentence has now been changed to "Efforts were made to minimize sample exposure to laboratory air by, for example, removing caps of sample vials only when reagents were added to the samples and just prior to sample analysis."

L187: *As this is the first mention of a table in the text, this should be Table 1.*

We have made the changes recommended by the reviewer (see above for table order).

Sections 2.3 and 2.4: *You use MQ and UHQ interchangeably throughout; please change all to UHQ (or MQ).*

We have made the changes recommended by the reviewer (changed all to UHQ)

L210-211: *You mention this later on L228 how the UHQ water was pH buffered; please specify that detail here as this is the first mention of pH buffered water.*

We have made the changes recommended by the reviewer.

L265: *Briefly summarize why d-DIHEN reduces memory effects, as you do for the ammonia injection.*

We have made the changes recommended by the reviewer on line 326.

L282: *Typo- NH3 subscript should be on δ11B*

We have made the changes recommended by the reviewer.

L280-285: *For comparison of the standard values, you should do a formal statistical test (t-test) for differences of averages. The d-DIHEN δ11B values for JCt are just barely within overlapping 2sd of the Gutjahr values and are probably significantly different with an alpha of 0.05.*

We have run a t-test comparing the samples to the inter-laboratory calibration study. Using a Ho of 16.98 for the samples by Gutjahr et al. (2014), the maximum end of the values for the inter-laboratory calibration study, the d-DIHEN values for JCt are *not* significant at a significance level of $p<0.05$ (p-value in this case is >0.06). However, while running the t-test, we did notice that the number of samples in Table 3 should have been 6 (instead of 12, and the 2SD should have been 0.69, instead of 0.6). We have changed these numbers in the table and in the text (and double checked that no other error was made). Note, that Gutjahr et al. (2014) also identifies that JCt did not reproduce as well as the JCp standard.

L312: *Again, a formal statistical test is needed here to support this assertion.*

See point above, although we will note in the text that significance was evaluated to be $p>0.05$.

L327: *Here you should say something like "Because our specimens come from only a single pH, we cannot constrain whether the relationship between δ11BCaCO3 and δ11Bborate significantly differs from unity, as is observed in other marine calcifiers (refs or cite intro section). Therefore, we assume that δ11BCaCO3 reflects only δ11Bborate, and thus that only borate ion is being incorporated into CaCO3. Given this assumption, the wide variation in δ11BCaCO3 ..."*

We have made the changes recommended by the reviewer. The text will now read as, after line 389: "…despite exposure of all species to approximately equivalent pH_{SW} of 8 (see Table 4). We cannot constrain whether the relationship between $\delta^{11}B_{CaCO3}$ and $\delta^{11}B$ of borate significantly differs from unity in this experiment with a single pH_{SW}, because $\delta^{11}B_{B(OH)4-}$ at the species' sites of calcification cannot be measured or calculated from the data at hand, it cannot be directly compared with the measured $\delta^{11}B_{CaCO3}$ to determine if $\delta^{11}B_{CaCO3}$ necessarily reflects calcifying fluid $\delta^{11}B_{B(OH)4-}$ and, thus, pH_{CF}. Assuming that only the borate ion is incorporated into biogenic $CaCO_3$ (i.e., $\delta^{11}B_{CaCO3}$ = calcifying fluid $\delta^{11}B_{B(OH)4-}$), the wide variation in $\delta^{11}B_{CaCO3}$ (*ca.* 20 ‰) amongst the investigated species reared under equivalent thermo-chemical conditions may indeed arise from inherent differences in pH_{CF} amongst the species."

L331-343: *Two items of note here:*

1) It is worth mentioning that Mavromatis and Noireaux experiments are from solutions with quite different chemistry than seawater, and thus the appropriateness of their conclusions for marine carbonates are still uncertain.

2) The B speciation from these experiments was derived via NMR. However, NMR only tells coordination state (tetrahedral/trigonal). Because there are multiple possible B incorporation pathways and B coordination (see/cite Balan et al., 2016), NMR cannot distinguish between boric acid and borate. On L 335, I would rephrase this to say "and coordination of B in inorganic CaCO3 (tetrahedral/trigonal ratio higher in aragonite than in calcite)". Then say that if coordination reflected the borate/boric acid ratio, aragonite-producing species should have a universally lower δ11B than calcite-producing species because δ11Bborate is always lower than δ11Bboric acid.

We have made the changes recommended by the reviewer. The text now reads as:

(1) Starting on line 406: "It should also be noted that these experiments (Mavromatis et al., 2015; Noireaux et al., 2015) analyzed carbonates precipitated from non-seawater solutions; therefore, further work is needed to determine the applicability of these findings to marine carbonates."

(2) Starting on line 404: "…and coordination of B in inorganic $CaCO_3$ (tetrahedral/trigonal ratio higher in aragonite than in calcite), B/Ca ratio alone does not appear to influence boron isotope fractionation in $CaCO_3$ (Noireaux et al., 2015)."

L367: *Same point on NMR being coordination; at best this may represent boric acid Incorporation AND* ***L373-376:*** *There it is! Excellent, move this discussion up to L331-343 area when you first discuss Mavromatis/Noireaux data, and then reference it again here.*

Although we agree with the statements made by the reviewer, we believe that the changes that we made above appropriately highlight the importance of the general discussion on **L373-376** and do not feel it is necessary to move the discussion up. We believe that the discussion is more relevant in the section on Coralline red alga.

Section 4.2.2 *: There are multiple instances where spaces are needed between words and at ends of sentences—check this.*

We have made the changes recommended by the reviewer.

Section 4.2.3*: Interesting. Without tooting my own horn here, I'd recommend mentioning that the relative $\delta_{11}B$ deviations are quite similar to that observed in the high-Mg calcite of bamboo corals by Farmer et al. 2015. Perhaps there is something systematic about $\delta_{11}B$ in HMC?*

We find the comment made by the reviewer to be intriguing, however, we do not think we have enough data to provide a solid argument suggesting a potential systematic relationship between $\delta^{11}B$ in HMC organisms, as the reviewer suggested. Especially considering that our calcareous red algae and serpulid worms are also HMC.

L404: *space between "initially" and "produce"*

We have made the changes recommended by the reviewer.

L425: *"which has been attributed to"*

We have made the changes recommended by the reviewer.

L504-509*: Here you say what you should in the introduction. Remove this from here and incorporate this into the intro.*

We have made the changes recommended by the reviewer.

Fig. 1 *caption: note typo on pKB*

We have made the changes recommended by the reviewer.

Fig. 2: *please mention why you chose these particular datasets to show; there are a lot more B isotope data available than just this figure. Also, please narrow the y-axis to between 10 and 40 per mil.*

We wanted to show the boron isotopic composition for some of the most studied marine biogenic carbonate archives including corals, foraminifera and bivalves. We also wanted to show that the data has been reported to follow different borate fractionation curves. Therefore, we chose 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. To clarify, we will add this explanation to the figure caption for Fig. 2.

Fig. 5*: Nice figure! Could you also draw a line for the oyster and temperate urchin back to δ11Bborate, and then down to pH?*

We have made the changes recommended by the reviewer.

In addition to the response to reviewers, we have also included a 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

Line 1 - added “divergent” to title

Lines 5-15 - changed contact details including corresponding email for Jill Sutton (Jill.Sutton@univ-brest.fr)

Lines 17-41 - changed order of sentences and language in abstract

Pages 2-3

Lines 45-60 – improved language for these sections and order of citations using the Biogeosciences format.

Lines 61-65 – moved text based on reviewer’s request regarding ***L44-48*** (see reply to reviewer comments). Some of the text was also improved for language.

Lines 66-91 – improved language for these paragraphs

Pages 3-4

Lines 91-94 – deleted and included parts of this section to lines 94-99. This was done in again in response to the reviewer’s request for ***L44-48*** (see above).

Lines 94-136 – see reply to reviewer comments regarding ***L71-85 (and including L44-48).***

Lines 137-149 – Changed order of citations using the Biogeosciences format.

Pages 5-6

Lines 150-220 – improved language for these sections and order of citations using the Biogeosciences format.

Pages 7-9

Lines 227-228 – see reply to reviewer comment regarding ***L170-171***

Lines 239-324 – improved language for these sections and order of citations using the Biogeosciences format.

Lines 324-328 – see reply to reviewer comment regarding ***L265***

Pages 10-12

Lines 330-390 - improved language for these sections

Lines 390-399 - see reply to reviewer comment regarding ***L327***

Lines 400-425 - improved language for these sections

Lines 426-440 – changed order of presentation by moving the Coralline red algae before the corals. Also added the scientific name of the organisms. We also added a little more detail on Coralline red algae (Lines 434-439).

Pages 13-18

Lines 442-544 - improved language for these sections and order of citations using the Biogeosciences format.

Lines 545-582 – We found this section to not be very clear and so we re-worked this section.

Lines 583-652 - improved language for these sections and order of citations using the Biogeosciences format.

Tables and Figures and author contributions

Made changes based on recommendations made by reviewer. Also made a few minor changes to Tables 1 and 3, Figure captions, and the author contribution section to improve the language of the manuscript. Figures 2 and 5 were revised and are attached to this document.

δ^{11}B as monitor of calcification site pH in divergent marine calcifying organisms

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Abstract. The boron isotope composition (δ^{11}B) of marine biogenic carbonates has been predominantly studied as a proxy for monitoring past changes in seawater pH and carbonate chemistry. However, a number of assumptions regarding chemical kinetics and thermodynamic isotope exchange reactions are required~~In order~~ to derive seawater pH from ~~skeletal~~ δ^{11}B from biogenic carbonates~~boron isotope ratio data, a number of assumptions related to chemical kinetics and themodynamic isotope exchange reactions are necessary~~. It is also probable that δ^{11}B of biogenic carbonate reflects seawater pH at the organism's site of calcification, which may or may not reflect seawater pH~~Furthermore, the boron isotope composition of biogenic carbonates ($\delta^{11}B_{CaCO3}$) is assumed to reflect the δ^{11}B of dissolved borate ($B(OH)_4^-$) in seawater~~. Here we report the development of methodology for measuring the δ^{11}B of~~in~~ biogenic carbonate samples at the multi-collector inductively coupled mass spectrometry facility at Ifremer (Plouzané, France) and the evaluation of $\delta^{11}B_{CaCO3}$ in a diverse range of marine calcifying organisms reared for 60 days in isothermal seawater (25°C) equilibrated with an atmospheric pCO_2 of *ca.* 409 µatm. Average $\delta^{11}B_{CaCO3}$ composition for all species evaluated in this study range from 16.27 ‰ to 35.09 ‰~~,~~ including, in ~~-~~decreasing order: coralline red alga *Neogoniolithion* sp. (35.89 ± 3.71 ‰); temperate coral *Oculina arbuscula* (24.12 ± 0.19 ‰), serpulid worm *Hydroides crucigera* (19.26 ± 0.16 ‰), tropical urchin *Eucidaris tribuloides* (18.71 ± 0.26 ‰), temperate urchin *Arbacia punctulata* (16.28 ± 0.86 ‰), and temperate oyster *Crassostrea virginica* (16.03 ‰).~~.~~ ~~We evaluated the $\delta^{11}B_{CaCO3}$ 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 pCO_2 of *ca.* 409 µatm. We~~ These results are discussed in the context of each species' proposed mechanism of biocalcification and other factors that could influence skeletal and shell δ^{11}B, including calcifying site pH, the proposed direct incorporation of isotopically enriched boric acid (instead of borate) into biogenic calcium carbonate, and differences in shell/skeleton polymorph mineralogy. We conclude that the large inter-species variability in $\delta^{11}B_{CaCO3}$ (*ca.* 20 ‰) and significant discrepancies between measured $\delta^{11}B_{CaCO3}$ and $\delta^{11}B_{CaCO3}$ expected from established relationships between abiogenic $\delta^{11}B_{CaCO3}$ and seawater pH arise primarily from fundamental differences in calcifying site pH amongst the different species. These results highlight the potential utility of δ^{11}B as proxy of calcifying site pH for a wide range of calcifying taxa and underscore the importance of using species-specific seawater pH-$\delta^{11}B_{CaCO3}$ calibrations when reconstructing seawater pH from δ^{11}B of biogenic carbonates.~~We discuss these results in the context of various proposed mechanisms of biocalcification, including the~~

~~potentially critical role that internal calcifying site pH plays in regulating CaCO$_3$ saturation state and borate δ^{11}B at the site of calcification and, thus, the δ^{11}B composition of calcifers' 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 the partial pressure of atmospheric CO$_2$ (pCO$_2$) have had on ~~marine~~ the carbonate chemistry of seawater. The recent anthropogenic increase in pCO$_2$ has already resulted in a significant decrease in seawater pH (Bates, 2007; Byrne et al., 2010; Dore et al., 2009; Feely et al., 2008; Feely et al., 2016; ~~Dore et al., 2009; Byrne et al., 2010;~~ Gonzalez-Davlia et al., 2010; IPCC, 2014~~; Feely et al., 2016~~), ~~with potential effects on~~which affects the ability of marine calcifying organisms to produce ~~skeletal~~ their shells and skeletons~~calcium carbonate~~ (CaCO$_3$; IPCC, 2014). ~~Ocean acidification~~Experimental studies have revealed that organismal responses to ocean acidification vary widely amongst taxa, highlighting the complexity of biological responses to ~~ocean acidification~~global change stressors (e.g., Kroeker et al., 2010; Kroeker et al., 2013; Ries et al., 2009~~; Kroeker et al., 2010; Kroeker et al., 2013~~) and necessitating a more ~~complete~~ thorough understanding of how an organism's mechanism of biocalcification governs its specific response to ocean acidification.

1.1 Theoretical model of δ^{11}B variation with pH

The boron isotope composition (δ^{11}B) of biogenic CaCO$_3$ (δ^{11}B$_{CaCO3}$) has been primarily used as a pal~~a~~eoceanographic 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; Trotter et al., 2011; Vengosh et al., 1991; Wei et al., 2009). 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 is~~behaves~~ conservatively distributed throughout the ocean (Foster et al., 2010)~~,~~ making δ^{11}B a potentially~~n~~ ~~an~~ attractive ~~palaeo~~ proxy for pal~~a~~eo-seawater pH. ~~The development of this proxy is based on a theoretical model of δ^{11}B variation with pH (Zeebe and Wolf-Gladrow, 2001) that assumes that δ^{11}B$_{CaCO3}$ reflects the δ^{11}B composition of borate in seawater (δ^{11}B$_{B(OH)4}$; Hemming and Hanson, 1992). The theoretical model of δ^{11}B variation as a function of pH requires knowledge of the fractionation factor (α) 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 vary as a function of ~~are~~ pH~~- dependent~~ (Fig. 1)~~, as defined by~~ pursuant to the following equilibrium reaction:

$B(OH)_3 + H_2O$ ↔ ~~⟷~~ $B(OH)_4^- + H^+$

In modern seawater, B(OH)$_4^-$ represents *ca.* ~~~~~24.15 % of dissolved boron~~ species~~, assuming that the dissociation constant (pK$_B$) between the~~se~~ two species of boron is 8.597 (at 25 °C, pH = 8.1, 35 psu; 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 in the ground state energy of molecular vibration of these chemical species in solution. The isotopic composition of boron is expressed following standard convention:

$$\delta^{11}B = [(^{11}B_{sample}/^{10}B_{sample})/(^{11}B_{standard}/^{10}B_{standard})-1] \times 1000\ (‰); \quad (1)$$

where the reference standard is NIST SRM 951 (Catanzaro et al, 1970).

The δ^{11}B of modern seawater is 39.61 ± 0.20 ‰ (Foster et al., 2010) and a large (> 20‰) and constant isotope fractionation ~~factor (α)~~ ~~α~~ exists between the two aqueous species described above. The fractionation factor (α) for~~α between~~ boric acid and borate ion is defined as:

$$\alpha \equiv \frac{(11B/10B)_{\text{Boric acid}}}{(11B/10B)_{\text{Borate ion}}};$$

A ~~wide~~ range of theoretical and empirical values for α has~~ve~~ been suggested (Byrne et al., 2006; Kakihana et al., 1977; Klochko et al., 2006; Nir et al., 2015; Palmer et al., 1987). For example, ~~α~~α of~~=~~ 1.0194 was calculated from theory by Kakihana et al. (1977) and was widely applied in ~~palaeo-~~reconstructions of pal~~a~~eo-seawater pH (Hönisch et al., 2004; Kakihana et al., 1977; Sanyal et al., 1995). Zeebe (2005) used analytical techniques and *ab initio* molecular orbital theory to calculate α ranging from 1.020 to 1.050 at 300 K. Zeebe (2005) provided several arguments in support of ~~α~~α ≥~~≧~~ 1.030, ultimately concluding that experimental work was required to determine the α for~~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 α (Klochko et al., 2006; Rustad and Bylaska, 2007). An empirical α 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 (Rollion-Bard and Erez, 2010; Xiao et al., 2014). Moreover, due to the ability of some calcifying organisms to alter carbonate chemistry at their site of calcification, ~~empirical species-specific calibrations between $\delta^{11}B_{CaCO3}$ and seawater pH (pH_{SW}) are likely more appropriate than theoretical αα 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).~~ paleo-seawater pH may not simply be reconstructed by projecting measured δ^{11}B of calcium carbonate~~ite~~ ($\delta^{11}B_{CaCO3}$) onto a theoretical seawater borate δ^{11}B ~~composition of borate in seawater~~ ($\delta^{11}B_{B(OH)4-}^{-}$)-pH curve (see also Anagnostou et al., 2012; Honïsch et al., 2003; Sanyal et al., 1996, Sanyal et al., 2001; ~~Honïsch 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 (pH_{SW}) and $\delta^{11}B_{CaCO3}$.

The~~ development of the~~ δ^{11}B-based paleo-seawater pH proxy ~~to date~~ is based on a theoretical model of δ~~^{11}B~~$^{11}B_{B(OH)4-}$ -variation with pH ~~(Zeebe and Wolf-Gladrow, 2001) that assumes that $\delta^{11}B_{CaCO3}$ reflects the δ^{11}B composition of borate in seawater ($\delta^{11}B_{B(OH)4-}$; (Hemming and Hanson, 1992) as given~~described by the following equation (Zeebe and Wolf-Gladrow, 2001):

$$pH = pK_B = -\log\left(\frac{\delta^{11}B_{CaCO3} - \delta^{11}B_{SW}}{(\delta^{11}B_{SW} - \alpha_B) \times (\delta^{11}B_{CaCO3} - \varepsilon_B)}\right)$$

T~~Uncertainties in t~~his theoretical model of $\delta^{11}B^{11}B_{B(OH)4-}$ variation as a function of seawater pH requires knowledge of the α for isotope exchange between the aqueous species of boron, the dissociation constant (pK_B), and the isotopic composition of total boron in seawater (Pagani et al., 2005)~~.)~~ —each of which can introduce uncertainty into the pH reconstruction.

Application of this proxy also assumes that $\delta^{11}B_{CaCO3}$ reflects seawater $\delta^{11}B_{B(OH)4-}$ and, thus, seawater pH (Hemming and Hanson, 1992). ~~Using $\delta^{11}B_{CaCO3}$ to derive pHsw requires knowledge of the relationship between $\delta^{11}B_{CaCO3}$ and $\delta^{11}B_{B(OH)4}^{-}$. One option is to~~ Although early studies assumed that $\delta^{11}B_{CaCO3}$ was indeed equivalent to seawater~~=~~ $\delta^{11}B_{B(OH)4}^{-}$~~ as was done by early studies~~ (e.g., Hemming and Hanson, 1992), ~~). However,~~ Sanyal et al. (2000,~~;~~ 2001) ~~pointed out~~observed that empirically derived $\delta^{11}B_{CaCO3}$-pH curves of biogenic and abiogenic calcites were parallel but vertically offset from the theoretical

$\delta^{11}B_{B(OH)4-}$-pH curve, which led them to conclude that paleo-seawater pH cannot always be directly calculated from $\delta^{11}B_{CaCO3}$ using the theoretical $\delta^{11}B_{B(OH)4-}$-pH relationship (i.e., $\delta^{11}B_{CaCO3}$-pH relationships must be empirically calibrated for the species hosting the paleo-pH proxy).

~~The most widely applied framework in which the boron isotope composition of carbonates is interpreted~~ The δ^{11}B-based paleo-seawater pH proxy also relies on the assumption that $B(OH)_4^-$ is the dominant species of dissolved inorganic boron incorporated into $CaCO_3$ minerals~~ as they~~ precipitated from seawater~~olution~~. It is ~~also~~ well established that δ^{11}B of dissolved $B(OH)_4^-$ is controlled by solution pH (c.f. Hemming and Hönisch, 2007; see discussion above). Therefore, $\delta^{11}B_{CaCO3}$ should reflect pH of the precipitating solution if $B(OH)_4^-$ is indeed the dominant species of dissolved inorganic boron incorporated into $CaCO_3$, which is consistent with ~~the observations of a number of~~numerous empirical studies (see Hemming and Hönisch, 2007, for summary).

More recently~~.~~, however, alternative models of boron incorporation into $CaCO_3$ have been proposed (Balan et al., 2016; Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015~~; Balan et al., 2016~~). ~~Generally speaking, theset~~These alternative models present a potential challenge to the utility of boron isotopes ~~to reconstruct~~in~~to~~ reconstructing calcifying fluid and paleo-seawater pH (Balan et al., 2016; Klochko et al., 2009 ; Mavromatis et al., 2015; ~~ ~~Noireaux et al., 2015; Uchikawa et al., 2015~~; Mavromatis et al., 2015; Balan et al., 2016~~). ~~They~~These studies~~y~~ present evidence consistent with the incorporation of boric acid, ~~alongside~~along with~~gside~~ borate, ~~incorporation~~ into some carbonates (~~eg.~~e.g. Noireaux et al., 2015; Uchikawa et al., 2015) and/or the ~~presence~~ occurrence of trigonal boron in the carbonate lattice due to transformation from borate during carbonate precipitation (e.g. Mavromatis et al., 2015). Some of these studies also ~~highlight~~ suggest that calcite ~~may be~~is more prone to ~~this complication~~boric acid incorporation than aragonite (e.g. Noireaux et al., 2015~~). Here~~)., H~~h~~owever, ~~as~~ these studies ~~precipitated~~ evaluated inorganic carbonates precipitates from fluids of compositions that differed substantially from ~~s with different compositions to~~ seawater; it is yet to be determined whether boric acid incorporation ~~it remains to be shown that this phenomena~~ is equally as prevalent in ~~marine~~ carbonates that are precipitated from seawater. ~~.~~ ~~Here~~Nevertheless, ~~here, as an alternative hypothesis to a primary pH control over biomineral δ^{11}B composition,~~ we ~~also consider~~evaluate ~~the compatibility of our data with the different models of boron incorporation~~boric acid incorporation as an alternative to our hypothesis that calcifying fluid pH exerts primary control over the δ^{11}B composition of most biogenic carbonates.~~.~~

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; Rollion-Bard et al., 2003, 2011b; Holcomb et al., 2010; Krief et al., 2010; Trotter et al., 2011; Ries, 2011a; Anagnostou et al., 2012; McCulloch et al., 2012; Wall et al., 2016), coralline red algae (Borowitzka and Larkum, 1987; McConnaughey and Whelan, 1997; Donald et al., 2017), calcareous green algae (~~De Beer and Larkum, 2001;~~ Borowitzka and Larkum, 1987; De Beer and Larkum, 2001; 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 $CaCO_3$ 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:

$$H_2CO_3 \leftrightarrow HCO_3^- + H^+$$

and

$$HCO_3^- \leftrightarrow H^+ + CO_3^{2-};$$

which are respectively governed by the following stoichiometric dissociation constants:

$$K^*_1 = [HCO_3^-][H^+]/[H_2CO_3]$$

and

$$K^*_2 = [CO_3^{2-}][H^+]/[HCO_3^-]$$

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_{CaCO3}) following:

$$\Omega_{CaCO3} = [Ca^{2+}][CO_3^{2-}]/K^*_{sp}$$

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.

The decrease in pH_{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 pH of their calcifying fluid (pH_{CF}), despite reduced external pH, should convert much of this increased DIC, occurring primarily as HCO_3^-, back into the CO_3^{2-} ~~that they need~~needed for calcification (Ries, 2011b , 2011a~~, 2011b~~; Ries et al., 2009). Thus, an organism's specific response to CO_2-induced ocean acidification should be~~is~~ critically dependent upon that organisms' ability to regulate pH at their site of calcification.

M~~It should be noted that m~~arine calcifiers biomineralize in diverse ways, with~~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., Al-Horani et al., 2003; Cohen and Holcomb, 2009; 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; Meibom et al., 2008; Tambutté 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., Ma~~i~~rie 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 primarily high-Mg calcite extracellularly, but within a chemically controlled ~~(and/or aragonite) crystals from an intercellular calcifying~~ fluid bound~~ed~~ by adjacent cells. ~~(Simkiss and Wilbur, 1989). Notably, biomineralization by coralline red algae occurs primarily within the cell wall and often has a preferred crystal orientation~~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).

Various mechanisms have been proposed for elevating pH_{CF}, 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_{CF} should strongly influence their ability to convert DIC into CO_3^{2-}, thereby impacting their specific calcification response to CO_2-induced ocean acidification.

1.3 Relationship between calcification site pH and $\delta^{11}B_{CaCO3}$

~~Organisms that precipitate $CaCO_3$ from a discrete calcifying fluid may record in their shells and skeletons $\delta^{11}B_{CaCO3}$ compositions that reflect pH_{CF} of their calcifying fluid (Farmer et al., 2015; Holcomb et al., 2014; Martin et al., 2016; McCulloch et al., 2012 Holcomb et al., 2014; Farmer et al., 2015; Martin et al., 2016).~~ Numerous studies have documented a systematic relationships between ~~the~~ pH_{SW} and the $\delta^{11}B_{CaCO3}$ composition of foraminiferal shells and coral skeletons (Fig. ~~2).~~2) that are generally consistent with theoretically derived relationships between seawater pH and $\delta^{11}B_{B(OH)4-}$~~.~~. However, the observed relationships between biogenic $\delta^{11}B_{CaCO3}$ and pH_{SW} vary widely amongst taxa (Fig. 2), and are generally ~~differ~~ offset 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 $CaCO_3$ (Noireaux et al., 2015; Sanyal et al., 2000~~; Noireaux et al., 2015~~).

One hypothesis for the discrepancies between the expected $\delta^{11}B_{CaCO3}$-pH relationship and those~~at~~ actually observed for biogenically precipitated $CaCO_3$ is that most marine calcifiers are not precipitating their $CaCO_3$ directly from seawater, but rather from a discrete calcifying fluid with a pH (pH_{CF})~~CF~~ that is substantially elevated relative to that of their external seawater~~.~~ (pH_{SW}~~w~~). ~~Prior~~ For example, prior studies have shown that~~,~~ for a given pH_{SW}, $\delta^{11}B_{CaCO3}$ of the coral species *Porites cylindrica* and *Acropora nobilis* are~~is~~ moderately elevated relative to $\delta^{11}B_{CaCO3}$ of the foraminifera *Globigerinoides sacculifer* and substantially elevated relative to the ~~mollusk~~ mussel *Mytilus edulis* (Fig. 2; Heinemann et al., 2012; Hönisch 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_{CF} of mussels. This is consistent with pH microelectrode (Al-Horani et al., 2003; Ries, 2011a), ~~boron isotope (e.g., Anagnostou et al., 2012; Krief et al., 2010; McCulloch et al., 2012; 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_{CF} to 8.5 – 10, versus their external pH_{SW} of 8, that foraminifera maintain their pH_{CF} between 8 and 9 (Jorgensen et al., 1985; Rink et al., 1998), and that bivalves maintain their pH_{CF} between 7.5 and 8 (Crenshaw, 1972). ~~Boron isotope data on aragonitic scleractinian corals is also broadly consistant with the idea of elevated pH_{CF} (e.g., Anagnostou et al., 2012; Krief et al., 2010; McCulloch et al., 2012; 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).~~

Here, we investigate differences in $\delta^{11}B_{CaCO3}$-pH relationships amongst taxonomically diverse biogenic calcification systems and discuss the compatibility of these observations with the hypothesis that $\delta^{11}B_{CaCO3}$ of biogenic carbonate is recording pH_{CF}, rather than pH_{SW}—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~~.~~ acidification. By systematically investigating the $\delta^{11}B_{CaCO3}$ 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 $\delta^{11}B_{CaCO3}$ composition of biogenic carbonates.

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 Spectrometrie Océan (PSO), Ifremer (Plouzané, France). Efforts were made to minimize sample exposure to laboratory air by,~~,~~ for example, ~~only~~ removing ~~(e.g. sample~~ caps of sample vials ~~were removed~~ only when reagents were added to the samples and just prior to sample analysis.~~).~~

2.2 Reagents

Ultra-pure reagents were used for all chemical procedures. The source of high-purity water (UHQ) for the procedures ~~is~~ was 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_4OH 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_4OH. 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

2.3.1 Samples

T~~In t~~his study~~, we~~ evaluated the $\delta^{11}B_{CaCO3}$ of 6 ~~highly~~ divergent species of marine calcifiers ~~that had been~~ 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 tropical coralline red alga (*Neogoniolithion sp.*), a tropical urchin (*Eucidaris tribuloides*), a temperate urchin (*Arbacia punctulata*), a serpulid worm (*Hydroides crucigera*), and ~~an American~~a temperate oyster (*Crassostrea virginica*; see Ries et al., 2009 for details). The specimens were subsampled for new growth relative to a barium marker emplaced at the start of the experiment (details in Ries, 2011), homogenized, and at least 3~~multiple~~ specimens per species analyzed for $\delta^{11}B_{CaCO3}$ ~~were evaluated (Table 3)~~.

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) for $\delta^{11}B$ ~~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) a laboratory coral standard (NEP; *Porites* sp.) 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~~ functions 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 ~~T~~t~~wo methods of B extraction are presented: ~~(~~batch and column chemistry.~~),~~ ~~where ~~For both, 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 ~~four biogenic $CaCO_3$ samples (*Porites* coral, temperate urchin, giant clam, American oyster).

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_4OH) H_2O_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 the acid was removed. The samples were washed twice with pH-buffered UHQ water (buffered to pH 7 with 2 % NH_4OH), centrifuged, and the water was removed. Dissolution of each sample was then performed by addition of 20 µL of 3 M HNO_3 followed by 300 µL of 0.05 M HNO_3. The pH of each sample was then adjusted to pH 7 with 0.2 M NH_4OH, 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 pH 7 prior to sample loading.

2.4.2 Column chemistry method

A column chemistry protocol for B extraction (described in ~~table ~~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 UHQ~~MQ~~-H_2O (pH=7), 0.5 M HNO_3, and again with pH-buffered UH~~M~~Q-H_2O. ~~The eluent was measured to ensure that it ~~After confirming that the eluent was at pH 7, t~~ prior to loading of the sample. T~~he 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, after which~~nd then~~ the B was eluted in 1000 µL of 0.5 M HNO_3. Column yields were greater than 95 % (Fig. 3) and elution tails of every sample were checked with an additional~~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 analysi~~e~~s by MC-ICPMS to verify ~~the ~~retention of B on the column and removal of other elements (e.g., Ca, Na, Ba, U).

2.4.3 Batch method

The batch method approach to B separation was conducted under closed conditions in ~~an attempt~~order 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 HNO_3, and then rinsed three times with 500 µL of UH~~M~~Q water ~~(buffered to pH 7 with 2 % NH_4OH)~~ ~~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 0.05 M HNO_3 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 HNO_3 to ensure that all of the B was recovered in the initial 500 µL 0.05 M HNO_3 solution.

2.5 Procedural blanks

The total yield of B from procedural blanks, which ~~encompasses~~ should reflect reagent, air-borne and procedural contamination, was sub-nanogram (lowest yields~~s~~ for column and batch methods were ~~=~~ 0.5 ng and~~and batch =~~ 90 pg, respectively). 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 $\delta^{11}B$ 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 HNO_3 for several minutes, the residual ^{11}B and ^{10}B signals were in the range of 10 – 80 mV, equivalent to 5 % (30 ppb) and 3 % (130 ppb), respectively (see Fig. S1 for ^{11}B 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 HNO_3. Each analysis consisted of a 2-minute simultaneous collection of masses 11 and 10 on Faraday cups H3 and L3 equipped with 10^{11} Ω resistors. Each sample was analysed in duplicate during a single analytical session, with replicate analyses not sharing a bracketing standard. T~~As such, t~~he boron isotope ratios are reported~~determined~~ as delta values ($\delta^{11}B$). The $\delta^{11}B$ of the calcium carbonate standards 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 their published reference values (Foster et al., 2013; McCulloch et al., 2014).

The MC-ICPMS ~~method~~ is ~~a~~ commonly used ~~approach~~ to measure $\delta^{11}B$ due to its capacity for rapid, accurate, and reproducible analyses (see McCulloch et al., 2014, for a recent 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 $\delta^{11}B_{CaCO3}$-based estimates of pH_{CF} to the analytical uncertainty cited above, two different injection methods (described below) were evaluated to determine ~~what method~~which is most suitable for minimizing analytical error.

2.7.1 Demountable direct injection nebulizer

Memory effects, as described above in section 2.7, were addressed by introducing samples to the plasma with a demountable direct injection high-efficiency nebulizer (d-DIHEN; Louvat et al., 2014). The d-DIHEN method minimizes the influence of memory effects by eliminating the use of a spray chamber and directly injecting the sample into the plasma (see Louvat et al., 2014, for details). 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 *ca.* ~~\~~~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

The yields for boron extraction for both methods were evaluated for various biogenic $CaCO_3$ samples and were typically between 97 and 102 % (determined by HR-ICPMS; see section 2.6). Washes with pH-buffered UH~~M~~Q-H_2O 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 $CaCO_3$ standards JCp-1 and JCt-1, a coral (*Porites* sp.; $\delta^{11}B_{NH3} = 24.45 \pm 0.28$ ‰, $\delta^{11}B_{d\text{-}DIHEN} = 24.30 \pm 0.16$ ‰) and a giant clam (*Tridacna gigas*; $\delta^{11}B_{NH3}$ = 16.65~~$_{NH3}$~~ ± 0.39 ‰, $\delta^{11}B_{d\text{-}DIHEN}$ = 17.5 ± 0.6~~0~~9 ‰), and their values established via inter-laboratory calibration ($\delta^{11}B = 24.36 \pm 0.51$ ‰, 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 $\delta^{11}B_{CaCO3}$ obtained via the batch and column chemistry methods for each of the biogenic $CaCO_3$ samples analysed.

3.2 Boron isotope composition of marine biogenic $CaCO_3$

Average $\delta^{11}B_{CaCO3}$ composition for all species evaluated in this study range from 16.27 ‰ to 35.09 ‰ (Table 3). The individual and average data are presented in Tables 3 and 4, respectively, 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). ~~-~~The coralline red alga *Neogoniolithion sp.* (35.89 ± 3.71 ‰; n = 3) exhibited the highest $\delta^{11}B_{CaCO3}$, 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~~ temperate oyster *C. virginica* (16.03 ‰; n = 1). Therefore, a range of *ca.* 20 ‰ in $\delta^{11}B_{CaCO3}$ was observed across all species evaluated in this study (Table 3 and 4). Notably, these are the first published $\delta^{11}B_{CaCO3}$ data for serpulid worm tubes and oysters.

3.3 Compatibility of the interspecific range of $\delta^{11}B_{CaCO3}$ with established seawater borate $\delta^{11}B$-pH relationships

Because the investigated species were cultured under relatively equivalent conditions (pCO_2 of 409 ± 6 µatm, 32 ± 0.2 psu, 25 ± 0.1 °C; see Ries et al., 2009), differences in pH_{SW} could not have been a significant driver of the observed interspecific variability in $\delta^{11}B_{CaCO3}$ (*ca.* 20 ‰; Tables 3 and 4). In order to evaluate this *ca.* 20 ‰ interspecific variability in $\delta^{11}B$, the data are plotted against measured pH_{SW} and graphically compared with theoretical borate $\delta^{11}B$-pH curves often used to interpret $\delta^{11}B_{CaCO3}$ data in the context of pH_{SW} (Fig. 4). Clear offsets from the seawater borate $\delta^{11}B$-pH curve (Klochko et al., 2006) can be observed for several of the species: the temperate coral (*O. arbuscula*) and coralline red alga (*Neogoniolithion* sp.) fall above the curve, the temperate urchin (*A. punctulata*) and American oyster (*C. virginica*) fall below the curve, and the tube of the serpulid worm (*H. crucigera*) and the tropical urchin (*E. tribuloides*) fall nearly on the curve (see Fig. 4 and Table 3). The interpretation of these offsets from the seawater borate $\delta^{11}B$-pH curve is discussed below.

4 Discussion

4.1 Appropriateness of method for analysing $\delta^{11}B_{CaCO3}$ in marine $CaCO_3$ 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 for 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 (p-value <0.05) difference in $\delta^{11}B_{CaCO3}$ composition of the samples (Table 3). The most effective method for minimizing memory effects in the MC-ICPMS analyses was found to be d-DIHEN (Louvat *et al.*, 2011). However, d-DIHEN 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-DIHEN 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~~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 following sample ~~that follows~~. Both the column and batch methods 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~~ lower risk of B contamination due to reduced contact time with air and the small volumes of both resin and acids (both potential sources of contaminan~~ts~~ation) used in the separation process.

4.2 The $\delta^{11}B_{CaCO3}$ compositions of a diverse range of marine calcifiers

The six species investigated exhibited a broad spectrum of $\delta^{11}B_{CaCO3}$ compositions, ranging from 16.03 ‰ to 35.89 ‰ (Table 4) ~~even though our~~despite exposure of all species ~~specimens were all exposed~~ to ~~a single pH condition of single~~approximately equivalent pH_{SW} ~~condition~~values of ~~~~~8 (see Table 4). ~~As such, we~~We cannot constrain whether the relationship between $\delta^{11}B_{CaCO3}$ and $\delta^{11}B$ of borate significantly differs from unity in this experiment with a single pH_{SW}.~~; , as is observed in other marine calcifiers (see Introduction). ThereforeTtherefore, we assume that $\delta^{11}B_{CaCO3}$ reflects only $\delta^{11}B$ of borate, and thus that only borate ion is being incorporated into $CaCO_3$. Given this assumption, the wide variation in $\delta^{11}B_{CaCO3}$.~~ Because $\delta^{11}B_{B(OH)4-}$ at the species' sites of calcification cannot be measured or calculated from the data at hand, it cannot be directly compared with the measured $\delta^{11}B_{CaCO3}$~~A~~ to determine if $\delta^{11}B_{CaCO3}$ necessarily reflects calcifying fluid $\delta^{11}B_{B(OH)4-}$ and, thus, pH_{CF}. Assuming that only the borate ion is incorporated into biogenic $CaCO_3$ ~~structures~~ (i.e., $\delta^{11}B_{CaCO3}$ = calcifying fluid $\delta^{11}B_{B(OH)4-}$), the wide variation in $\delta^{11}B_{CaCO3}$ (*ca.* 20 ‰) amongst the investigated species~~,~~ reared under equivalent thermo-chemical conditions~~,~~ may indeed arise from inherent differences in pH_{CF} amongst the species. If this is the case, then the observed range in $\delta^{11}B_{CaCO3}$ amongst the species (16.03 ‰ to 35.89 ‰) translates to an approximate range in pH_{CF} of 7.9 – 9.4.

The amount of b~~B~~oron (i.e., B/Ca) co-precipitated~~ion~~ with inorganic (i.e., abiogenic) $CaCO_3$ is known to be dependent on solution pH and inorganic $CaCO_3$ precipitation rate. 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 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~~ coordination of B in inorganic $CaCO_3$ (tetrahedral/trigonal ~~borate/boric acid~~ ratio higher in aragonite than in calcite), B ~~incorporation~~/Ca ratio alone does not appear to influence boron isotope fractionation in $CaCO_3$ (Noireaux et al., 2015). ~~Note that~~It should also be noted that ~~-~~these experiments ~~conducted by~~ (Mavromatis et al., ~~(~~2015; ~~) and~~ Noireaux et al., ~~(~~2015) analyzed carbonates precipitated from ~~used~~ non-seawater solutions ~~that have a different chemistry to that of seawater~~; therefore, further work is needed to determine ~~,~~ the applicability of these findings to marine carbonates. Furthermore, because the borate/boric acid ratio is higher in aragonite than in calcite, aragonite-producing species (corals, serpulid worms) should have a universally lower $\delta^{11}B_{CaCO3}$ composition than calcite-producing species (urchins, coralline algae, oysters) if shell mineralogy was the primary driver of the observed interspecific variation in $\delta^{11}B_{CaCO3}$ compositions – a trend that is not observed (Fig. 4).~~-~~ Thus, interspecific differences in polymorph mineralogy cannot, alone, explain the species' disparate $\delta^{11}B_{CaCO3}$ compositions. The more parsimonious explanation for these observed differences in $\delta^{11}B_{CaCO3}$ appears to be differences pH_{CF}, 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 isotopic compositions (e.g., O, C, B) of biogenic marine $CaCO_3$ (e.g., Hemming and Hanson, 1992; McConnaughey, 1989). Notably, many marine calcifiers exhibit ~~have~~ $\delta^{11}B_{CaCO3}$ ~~values~~ that differs from ~~the~~ ~~$\delta^{11}B_{B(OH)4-}$~~ ~~$\delta^{11}B$ composition of borate ions dissolved in seawater at an equivalent pH~~of their surrounding seawater (Figs. 3 and 5). When interpreted in the context of the framework that skeletal $\delta^{11}B$ reflects pH_{CF} rather than the organism's ambient pH_{SW} ~~of the organism's surrounding seawater~~, these results suggest that marine calcifiers are precipitating their $CaCO_2$ from a discrete fluid with a pH_{CF} higher than, equal to, or, for some species, below that of seawater. A second hypothesis is that ~~whilst seawater~~ pH_{CF} exerts ~~some control~~some control over $\delta^{11}B_{B(OH)4-}$ ~~borate $\delta^{11}B$~~ at the site of calcification and, hence, $\delta^{11}B_{CaCO3}$, but that there are other species-specific effects that ~~may~~ also influence $\delta^{11}B_{CaCO3}$ composition. The compatibility of these two hypotheses with existing models of biomineralization and observed $\delta^{11}B_{CaCO3}$ for the various marine calcifiers investigated in the present study are discussed below.

~~4.2.1 Temperate coral~~

~~The average $\delta^{11}B_{CaCO3}$ for the temperate coral *O. arbuscula* evaluated in this study (24.12 ± 0.19 ‰; n = 3; Tables 3 and 4) is consistent with previously published values for aragonitic corals (Table 5; see references therein). Generally, aragonitic corals are enriched in ^{11}B when compared with a theoretical borate $\delta^{11}B$-pH curve (see Figures 2 and 4). The main vital effect typically used to describe ^{11}B-enrichment in corals, relative to seawater, is an increase in pH_{CF} at the coral's site of calcification (e.g., Anagnostou et al., 2012; McCulloch et al., 2012; 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) and pH-sensitive fluorescent dyes (Venn et al., 2009, 2011, 2013).~~

4.2.1~~2~~ Coralline red alga (*Neogoniolithon* sp.)

Coralline red algae are also thought to precipitate primarily high-Mg calcite from a calcifying fluid bounded by adjacent cells (Simkiss and Wilbur, 1989). Thus, biomineralization by coralline red algae occurs extracellularly but primarily within a chemically controlled environment within and adjacent to cell walls, with calcite crystals exhibiting preferred orientations—atypical of calcifying macroalgae (Simkiss and Wilbur, 1989). The average $\delta^{11}B_{CaCO3}$ for the branching, non-articulated coralline red alga *Neogoniolithion* sp. evaluated in this study (35.89 ± 3.71 ‰; n = 3; Tables 3 and 4) is higher than the $\delta^{11}B_{CaCO3}$ composition of any other calcifying marine organism evaluated to date (Table 5). Of particular interest, one of the coralline red alga specimens evaluated in this study exhibited $\delta^{11}B_{CaCO3}$ (39.94 ‰, Table 3) similar to the average $\delta^{11}B$ of the total dissolved boron in seawater~~$_{SW}$~~ (i.e., comprising the $\delta^{11}B$ composition of both dissolved borate and boric acid; 39.61 ‰) determined by Foster et al.~~,~~ (2010), raising the possibility that coralline red algae 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_{SW} of 8.1, the $\delta^{11}B_{CaCO3}$ compositions observed for the coralline algae in the present study would require incorporation of both inorganic species of boron at $[B(OH)_3]:[B(OH)_4^-]$ ratios of *ca.* 3:1~~75:25~~, which is not consistent with prior observations for inorganic and ~~organic~~ biogenic calcite. For example, ~~(e.g.,~~ Cusack et al., 2015, reported 30% trigonal boron in the calcite lattice of a different species of coralline alga~~e)~~. Therefore, boric acid incorporation ~~cannot~~ alone cannot ~~rule out pH_{CF} as a potential driver of~~explain ~~-~~the anomalously elevated $\delta^{11}B_{CaCO3}$ observed here for coralline algae (see also discussion in Donald et al., 2017).~~).~~ 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~~ the B ~~is indeed directly incorporated into~~in the calcite lattice of coralline algal skeleton is indeed incorporated directly as trigonal boron, as reported by Cusack et al.~~,~~ (2015), pH_{CF} would still need to be as high as 9 to explain the anomalously high $\delta^{11}B_{CaCO3}$ (see Fig. 5). Short et al.~~,~~ (2015) observed that epiphytic turf algae can increase pH_{SW} up to 9 within their diffusive boundary layer ~~above coralline algal crusts~~, driven by the algae's photosynthetic drawdown of aqueous CO_2, lending further support to the idea that other types of algae, such as coralline red algae, could maintain their calcifying fluid ~~near~~ at or above pH 9. Thus, $\delta^{11}B_{CaCO3}$ 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.

4.2.2 Temperate coral (*O. arbuscula*)

The average $\delta^{11}B_{CaCO3}$ for the temperate coral *O. arbuscula* evaluated in this study (24.12 ± 0.19 ‰; n = 3; Tables 3 and 4) is consistent with previously published values for aragonitic corals (Table 5; see references therein). Generally, aragonitic corals are enriched in ^{11}B when compared with a theoretical borate $\delta^{11}B$-pH curve (see Figures 2 and 4). The main vital effect typically used to explain ^{11}B-enrichment in corals, relative to seawater, is an increase in pH at the coral's site of calcification (e.g., Anagnostou et al., 2012; McCulloch et al., 2012; Rollion-Bard et al., 2011b; Trotter et al., 2011; 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) and pH-sensitive fluorescent dyes (Venn et al., 2009, 2011, 2013). The $\delta^{11}B$ of the coral's skeleton is not sufficiently high so as to be consistent with incorporation of significant boric acid into the coral's aragonite lattice.

4.2.3 Tropical and temperate urchins (*E. tribuloides, A. punctulata*)

The average $\delta^{11}B_{CaCO3}$ for the tropical urchin *E. tribuloids* (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, which were both ~~and~~ reared at equivalent seawater conditions (pH_{SW} = 8.0; 25 °C; 32 psu; Table 4), are lower~~less~~ than $\delta^{11}B_{CaCO3}$ previously reported for other echinoid species (see Table 4; 22.7 ‰ - 22.8 ‰), but are close to theoretical values of dissolved borate at the same~~ose~~ pH_{SW} ~~seawater conditions~~ (17.33 ‰; Fig. 4). Microelectrode evidence suggests that urchins calcify from fluids with a pH_{CF} and composition similar to that of seawater (Stumpp et al., 2012), which is supported by our observation that urchin $\delta^{11}B_{CaCO3}$ is similar to $\delta^{11}B$ of dissolved borate. The difference between the $\delta^{11}B_{CaCO3}$ of these two species of urchin and the theoretical value of $\delta^{11}B$ for seawater borate (17.33 ‰) is +1.38 ‰ for the tropical urchin and -1.05 ‰ for the temperate urchin – a difference that exceeds their inter-specimen 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 $\delta^{11}B_{CaCO3}$ by adjusting pH of their calcifying environment by only ± 0.1 unit~~s~~ (e.g., pH_{CF} 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 $\delta^{11}B_{CaCO3}$ 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_{CF} is lower than that of seawater. T~~Note that t~~he relatively low $\delta^{11}B$ ~~values for~~ of the urchins' tests ~~these high-Mg calcite-precipitating organisms are also~~is not consistent with the hypothesis that significant boric acid is incorporated~~ion~~ into the ~~carbonate lattice~~urchins' high-Mg calcite lattice (Fig. 5).

4.2.4 Serpulid worm tube *(H. crucigera)*

The average $\delta^{11}B_{CaCO3}$ 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 the theoretical value of $\delta^{11}B$ for seawater borate (Fig. 4). The serpulid worm *H. crucigera* ~~secretes~~ produces ~~their~~ its calcareous tube from a combination of aragonite and high-Mg calcite (HMC; Ries, 2011b). 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 (Hedley, 1956). The samples of *H. crucigera* evaluated in this study were exposed to environmental conditions (pH_{SW} = 8.1; 25 °C; 32 psu; Table 4) yielding a theoretical seawater $\delta^{11}B_{B(OH)4-}$ ~~borate $\delta^{11}B$ and, thus, $\delta^{11}B_{CaCO3}$~~ of 18.38 ‰, which is 0.88 ‰ less than $\delta^{11}B_{CaCO3}$ measured for ~~*H. crucigera*~~this species. Similar to the tropical urchin discussed above, the serpulid worm could generate this divergence in $\delta^{11}B_{CaCO3}$ from seawater $\delta^{11}B_{B(OH)4-}$ ~~borate $\delta^{11}B$~~ by elevating pH_{CF} by 0.08 units relative to pH_{SW}. The relatively low $\delta^{11}B$ of the serpulid worm tube is not consistent with significant boric acid incorporation into the worm's calcite and aragonite lattices (Fig. 5). 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. To our knowledge, these are the first reported 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 *(C. virginica)*

The $\delta^{11}B_{CaCO3}$ for the American oyster *C. virginica* evaluated in this study (16.03 ‰; n = 1; Tables 3 and 4) is less than the theoretical value of seawater $\delta^{11}B_{B(OH)4-}$ ~~$\delta^{11}B$ for seawater borate~~ at equivalent pH_{SW}(Fig. 4). Oysters construct their shells of LMC (aragonite during the larval stage) from a discrete calcifying 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., Marie et al., 2012; Mount et al., 2004; Weiner et al., 1984; Wheeler 1992; Wilbur and Saleuddin 1983~~; Wheeler 1992; Marie et al., 2012; Weiner et al., 1984; Mount et al., 2004).~~). The specimens of~~ample of~~ *C. virginica* evaluated in this study ~~was exposed to~~were grown in seawater conditions (pH_{SW} = 8.2; 25 °C; 32 psu; Table 4) that yield a theoretical seawater $\delta^{11}B_{B(OH)4-}$ ~~borate $\delta^{11}B$, and thus $\delta^{11}B_{CaCO3}$,~~ of 19.57 ‰, which is 3.54 ‰ greater than $\delta^{11}B_{CaCO3}$ measured for this species ~~*C. virginica*~~. The observation that oyster $\delta^{11}B_{CaCO3}$ is substantially less than seawater $\delta^{11}B_{B(OH)4-}$ ~~theoretical $\delta^{11}B$ of seawater borate~~ suggests that pH_{CF} of oyster extrapallial fluid 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 has been ~~the author~~ attribute~~s~~d 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., Addadi et al., 2003; Marie et al., 2012; ~~Addadi et al., 2003;~~ Weiner et al., 1984) and/or maintaining elevated levels of dissolved inorganic carbon within the EPF. The oyster could generate this negative divergence in $\delta^{11}B_{CaCO3}$ from seawater borate $\delta^{11}B$ by decreasing pH_{CF} by 0.35 units (Table 4), which, given the proximity of the independent pH-microelectrode measurements of oyster EPF, seems to be a plausible explanation for why oyster $\delta^{11}B_{CaCO3}$ falls below the theoretical seawater $\delta^{11}B_{B(OH)4-}$ ~~borate $\delta^{11}B$~~-pH curve (Klochko et al., 2009; Fig 5). The relatively low $\delta^{11}B$ of the oyster calcite is not consistent with significant boric acid incorporation into the oyster's calcite lattice (Fig. 5). To ~~our~~ the authors' knowledge, these are the first ~~reported~~ B isotope ~~measurements~~ analyses reported for oysters~~ and the $\delta^{11}B$ values for this low Mg calcite-precipitating organism are not consistantconsistent with significant boric acid incorporation into the carbonate lattice (Fig. 5).~~.

4.3 Estimating pH_{CF} from $\delta^{11}B_{CaCO3}$

The six~~6~~ species of calcifying marine organisms investigated in the present study exhibited average $\delta^{11}B_{CaCO3}$ ~~compositions~~ ranging from 16.27 ‰ to 35.09 ‰ (Table 3). Given that all six~~6~~ species were grown under nearly equivalent controlled laboratory conditions, the large interspecific range in $\delta^{11}B_{CaCO3}$ supports the hypothesis that $\delta^{11}B_{CaCO3}$ of biogenic carbonates is not simply inherited from $\delta^{11}B_{B(OH)4-}$ of the organism's surrounding seawater ~~boron isotope fractionation in marine calcifiers cannot be explained solely by borate incorporation at ambient pH_{SW}~~ (see Table 5 and references therein). Rather, we assert~~We hypothesize~~ that this species-dependent variability in $\delta^{11}B_{CaCO3}$ is driven by interspecific differences in the organisms' pH_{CF}. To explore this ~~hypothesis~~assertion, $\delta^{11}B_{CaCO3}$ values were converted to pH_{CF} from measured seawater temperature, salinity, ~~seawater~~ a total dissolved boron $\delta^{11}B$ value of 39.61 ± 0.20 ‰ (Foster et al., 2010), and an α of 1.0272 (Klochko et al., 2006; Table 4). In the absence of ~~empirical~~ direct measurements of calcifying fluid temperature, salinity, and total dissolved boron $\delta^{11}B$, these parameters are assumed to ~~reflect~~ be equivalent to those of the organism's surrounding seawater. Assuming that only borate is incorporated into the organisms' shells and skeletons (see Table 4), t~~T~~hese calculations yield a pH_{CF} (in order of decreasing magnitude) ~~, assuming that only borate is incorporated (see Table 4),~~ of ~~8.5 for the temperate coral (*O. arbuscula*),~~ 9.4 for the coralline red alga (*Neogoniolithion* sp.), 8.5 for the temperate coral (*O. arbuscula*), 8.2 for the serpulid worm (*H. crucigera*), 8.1 for the tropical urchin (*E. tribuloides*), and 7.9 for the temperate urchin (*A. punctulata*)~~, 8.2 for the serpulid worm (*H. crucigera*),~~ and ~~7.9 for the~~ American oyster (*C. virginica*).

4.3.1 ~~Sensitivity of~~Nonlinearity of $\delta^{11}B_{CaCO3}$-pH_{CF} relationship relative to ~~based calculations of pH_{CF} to choice of~~ pK_B ~~and α~~

~~Here, we evaluate the sensitivity of calculating pH_{CF} from measured $\delta^{11}B_{CaCO3}$ composition by testing the two principal factors that may influence the theoretical model of borate $\delta^{11}B$ variation as a function of both pH_{CF} and pH_{SW}; namely pK_B and α. 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.~~

The determination of pH_{CF} from pK_B, $\delta^{11}B$ of calcifying fluid ($\delta^{11}B_{CF}$), and $\delta^{11}B_{CaCO3}$ can be summarized with the following equation (Eq. 1):

$$pH_{CF} = pK_B - \log\left((\delta^{11}B_{CF} - \delta^{11}B_{CaCO3}) / (\delta^{11}B_{CF} - (\alpha \times \delta^{11}B_{CaCO3}) - 1000(\alpha - 1))\right); \quad (1)$$

where pK_B is 8.6152 (at 25°C and 32 psu; Dickson, 1990), $\delta^{11}B_{CF}$ is 39.61 ‰ (inherited from $\delta^{11}B_{SW}$; Foster et al., 2010), and α is 1.0272 (Klochko et al., 2006). Thus, Eq. 1 allows for the calculation of~~,~~ $\delta^{11}B_{CaCO3}$~~, can be calculated~~ across a range of pH_{CF} (Fig. 1b; Table S1).

~~It is important to note that the difference in $\delta^{11}B_{CaCO3}$ between each pH unit (when fluid pH < pK_B) 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 $\delta^{11}B_{CaCO3}$ difference of 0.35 ‰ (15.77 ‰ – 15.42 ‰), whereas a change in pH from 8.35 to 8.40 predicts a $\delta^{11}B_{CaCO3}$ difference of 0.74 ‰ (22.59 ‰ – 21.85 ‰). Thus, the relationship between pH_{CF} and $\delta^{11}B_{CaCO3}$ is nonlinear over the range of pH_{CF} of interest (7 < pH < 10), with pH having the greatest influence on $\delta^{11}B_{CaCO3}$, 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 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 t~~The sensitivity ~~analysis for the~~of $\delta^{11}B_{CaCO3}$ ~~derived determinations of~~ to changes in pH_{CF} increases ~~at a pK_B of 8.6152 indicates that a small change in pH_{CF} greatly influences $\delta^{11}B_{CaCO3}$, especially~~ as pH_{CF} approaches pK_B (8.6152; Table S1)~~)~~.). For example, a change in pH_{CF} from 7.75 to 7.80 predicts a $\delta^{11}B_{CaCO3}$ difference of 0.35 ‰ (15.77 ‰ – 15.42 ‰), whereas a change in pH from 8.35 to 8.40 predicts a $\delta^{11}B_{CaCO3}$ difference of 0.74 ‰ (22.59 ‰ – 21.85 ‰). Thus, the relationship between pH_{CF} and $\delta^{11}B_{CaCO3}$ is nonlinear over the pH_{CF} range of interest (7 < pH < 10), with pH having the greatest influence on $\delta^{11}B_{CaCO3}$ as fluid pH_{CF} approaches pK_B.

Fortuitously, the calcifiers investigated in the present study maintain their pH_{CF} within approximately 1 pH unit of pK_B (i.e., over the interval where small differences in pH_{CF} cause relatively large differences in $\delta^{11}B_{CaCO3}$). Therefore, for these organisms, it will be easier to obtain precise measurements of expected differences in $\delta^{11}B_{CaCO3}$ and, thus, differences in pH_{CF}. Conversely, it will be harder to obtain precise measurements of the differences in $\delta^{11}B_{CaCO3}$ (and pH_{CF}) for calcifiers that maintain their pH_{CF} more distal from pK_B—if such calcifiers indeed exist.

Along these same lines, slight differences in pH_{SW} of the experimental treatments (also proximal to pK_B) could conceivably translate to relatively large changes in $\delta^{11}B_{B(OH)4-}$ amongst the species' seawater treatments and, thus, their calcifying fluid $\delta^{11}B_{B(OH)4-}$ and $\delta^{11}B_{CaCO3}$. However, ~~,~~ the small range of ~~the organisms' seawater~~ pH_{SW} for the different species' experimental treatments (8.0-8.2; Table 4) could only account for a 2.24 ‰ range in $\delta^{11}B_{CaCO3}$ (Table S1), far less than the *ca.* 20 ‰ range that was observed amongst the different species in the present study. It therefore follows that the large variability in $\delta^{11}B_{CaCO3}$ (*ca.* 20 ‰) observed for the investigated species requires an alternative explanation, such as fundamental ~~changes~~ differences in their pH_{CF}.~~—particularly for the coralline alga, coral and oyster species that exhibited such large deviations in predicted vs. observed $\delta^{11}B_{CaCO3}$ (see section 4.2).~~

4.3.2 Sensitivity ~~analysis~~ of $\delta^{11}B$-derived pH_{CF} to choice of α

As discussed in the Introduction (section 1.1), much work has gone into establishing an α that accurately describes the pH-dependent relationship between $\delta^{11}B$ of dissolved borate and boric acid in seawater (see Xiao et al., 2014, for detailed discussion), with the earliest published pal~~a~~eo-pH reconstructions using a theoretical value of 1.0194 (Kakihana et al., 1977; see Fig. 2). An empirical α of 1.0272 (Klochko et al., 2006) has now been shown to better predict $\delta^{11}B_{B(OH)4-}$, viz. $\delta^{11}B_{CaCO3}$, across the~~a~~ range of pH relevant for seawater (Rollion-Bard and Erez, 2010; Xiao et al., 2014). However, $\delta^{11}B_{CaCO3}$ of many species of calcifying marine organisms fall either above or below ~~the~~ theoretical $\delta^{11}B_{B(OH)4-}$-pH_{SW} curves. It has long been suggested (and shown for corals) that calcifying organisms diverge from the predicted $\delta^{11}B_{CaCO3}$ 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; McCulloch et al., 2012; 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 $\delta^{11}B_{CaCO3}$ from the theoretical $\delta^{11}B_{B(OH)4}$~~-~~-pH_{SW} curves are interpreted as evidence of the differing capacities of calcifying marine species to modify pH_{CF}. Importantly, existing models of biomineralization for each species are generally compatible with these $\delta^{11}B_{CaCO3}$-derived estimates of pH_{CF} (see section 4.2).

Although an α of 1.0272 (Klochko et al., 2006) was used in the present study to estimate pH_{CF}, other theoretical values for α, yielding slightly different $\delta^{11}B_{B(OH)4}$-pH_{SW} ~~borate $\delta^{11}B$-pH~~ curves (e.g., Byrne et al., 2006; Palmer et al., 1987; see Fig. 4), will yield slightly different estimates of pH_{CF} for each organism. For example, using α 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 $\delta^{11}B_{CaCO3}$ of 24.12 ‰ (temperate coral; pH_{SW} = 8.1) yields pH_{CF} of 8.7, 8.6, 8.5, and 8.1, respectively—~~—~~a ~~difference~~ range of 0.6 pH units. It should also be noted that the lower the $\delta^{11}B_{CaCO3}$, the more sensitive the reconstructed pH is to choice of α. For example, changing α from 1.0272 to 1.0330 will result in a 0.24 pH unit shift for $\delta^{11}B_{CaCO3}$ of ~~=~~20 ‰, but only a 0.12 and 0.08 pH unit shift for $\delta^{11}B_{CaCO3}$ of~~=~~ 30 ‰ and 39.5 ‰, respectively. This underscores the importance of using the same α when comparing $\delta^{11}B_{CaCO3}$-based estimate of pH_{CF} amongst species.

4.3.3 Implications of $\delta^{11}B_{CaCO3}$-derived estimates of pH_{CF} for species-specific vulnerability to ocean acidification

~~Understanding~~ Establishing 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). Although it is widely known that many species of marine calcifiers promote calcification by raising pH at their site of calcification, the present study identifies the degree to which this strategy for biocalcification is employed across a range of divergent taxa. Marine calcifiers that employ this strategy for calcification may be more resilient to the effects of ocean acidification because their high pH_{CF} (relative to pH_{SW}) would cause HCO3- (elevated due to increased pCO_2) to dissociate into CO_3^{2-} for calcification, helping the organism to maintain an elevated Ω at its site of calcification (Ries et al., 2009). E~~Notably,~~valuation of this hypothesis in the context of the results of the present study shows that, indeed, the different species' $\delta^{11}B_{CaCO3}$ and ~~reconstructed~~ calculated pH_{CF} 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 $\delta^{11}B_{CaCO3}$ and, thus, pH_{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 $\delta^{11}B_{CaCO3}$ and, thus, pH_{CF} close to that of pH_{SW}. These results support the assertion that interspecific differences in pH_{CF} ~~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, and vulnerability to ocean acidification ~~within~~ of marine calcifying organisms.

~~4.3.4 Further calibration of the $\delta^{11}B_{CaCO3}$-derived determinations of pH~~

~~The observed deviations of the investigated species' $\delta^{11}B_{CaCO3}$ 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 $\delta^{11}B_{CaCO3}$ onto a theoretical seawater $\delta^{11}B_{B(OH)4}$-pH curve (see also Sanyal et al., 1996, Sanyal et al., 2001; Honïsch 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 $\delta^{11}B_{CaCO3}$.~~

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-DIHEN vs. ammonia addition), causes a significant difference in the measured $\delta^{11}B_{CaCO3}$ ~~composition~~ of the evaluated samples and standards. The batch method of boron extraction is ~~preferred~~ identified as preferable to ~~over~~ the column chemistry method ~~since~~ because 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 $\delta^{11}B_{CaCO3}$ ~~composition~~ of six species of marine calcifiers that were all grown under equivalent seawater conditions. The coralline red alga *Neogoniolithion* sp. (35.89 ± 3.71 ‰; n = 3) exhibited the highest $\delta^{11}B_{CaCO3}$, 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~~ temperate oyster *C. virginica* (16.03 ‰; n = 1). The observed *ca.* 20 ‰ range in $\delta^{11}B_{CaCO3}$ composition of the investigated species constitutes the largest range in biogenic $\delta^{11}B_{CaCO3}$ reported to date.

Consideration of these extreme interspecific differences in $\delta^{11}B_{CaCO3}$ in the context of existing models of biomineralization for the investigated species, combined with published measurements of pH_{CF} 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' $\delta^{11}B_{CaCO3}$ and ~~reconstructed~~ calculated pH_{CF} 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_{CF}. These observations contribute to the growing body of work that uses $\delta^{11}B_{CaCO3}$ as a tool to advance understanding of the mechanisms by which marine calcifiers build and maintain their shells and skeletons and, ultimately, how these organisms~~y~~ will respond to anthropogenic CO_2-induced ocean acidification.

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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.

Step	mg resin	15	62.5	125
1	Resin (uL)	60	250	500
2	UH~~M~~Q H2O at pH 7 (mL)	5	5	5
3	0.5 N HNO3 (mL)	2.5	2.5	5
4	UH~~M~~Q H2O at pH 7 (mL) x3	2.5	2.5	5
5	Check pH			
6	Sample Load (ng)	536	536	536
7	UH~~M~~Q H2O at pH 7 (mL) x3	1	1	2
10	Check pH			
11	0.05N HNO3 (mL)	0.5	0.5	0.5
22	UH~~M~~Q H2O at pH 7 (mL)	2	2	2

Table 2. Mass spectrometer operating conditions.

	d-DIHEN	**Ammonia Addition**
Injection System	Demountable Direct Injection High-efficiency Nebulizer	PFA teflon spray chamber with ESI PFA teflon 50 µL min^{-1} nebuliser
Sample Gas Flow Rate	0.3 L min^{-}	1.1 L min^{-1}
Running Concentrations	B = 50 ppb	B = 30 – 50 ppb (evaluated 30, 65, 130 ppb)
Sensitivity	35 V ppm^{-1}, total B	20 V ppm^{-1}, total B
Blank Level	< 0.5 % of ^{11}B signal after 30s in 2 % HNO_3, 0.1 % after 120s	< 5 % of ^{11}B signal after 30s in 0.05% HNO_3, 3% after 120s
Resolution	Low	Low
Forward Power	1200 W	1200 W
Accelerating Voltage	10 kV	10 kV
Plasma Mode	Wet Plasma	Wet Plasma
Cool Gas Flow Rate	16 L min^{-1}	16 L min^{-1}
Auxiliary Gas Flow Rate	0.9 L min^{-1}	0.9 L min^{-1}
Sampler Cone	Standard Ni cone	Standard Ni cone
Skimmer Cone	X Ni cone	X Ni cone
Interferences	^{40}Ar^{++++} ^{20}Ne^{++} resolved	^{40}Ar^{++++} ^{20}Ne^{++} resolved
Accuracy	0.2 ‰, 2sd, n = 6	0.2 ‰, 2sd, n = 6
Acquisition	30 x 4s	30 x 4s
Baselines	Counting times of 20 s	Counting times of 20 s

Table 3. Boron isotope composition ($\delta^{11}B$; ‰) of all species evaluated, including international carbonate standards JCp-1 (coral, *Porites* sp.) and JCt-1 (giant clam, *Tridacna gigas*~~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.

Sample type	**Name**	**$\delta^{11}B$**	**(2SD)**	***n***	**Cleaning**	**Separation**	**Injection**
Giant~~Hard~~ clam	JCt-1	17.50	0.69~~0~~	6	Ox	batch	d-DIHEN
Giant~~Hard~~ clam	JCt-1	16.90	0.30	6	Ox	batch	NH_3
Giant~~Hard~~ clam	JCt-1	16.34	0.64	2	Ox	column	NH_3
Giant~~Hard~~ clam	JCt-1	16.24	0.42	2	U	batch	NH_3
Porites coral	JCp-1	24.52	0.34	6	Ox	column	NH_3
Porites coral	JCp-1	24.30	0.16	10	Ox	batch	d-DIHEN
Porites coral	JCp-1	24.65	0.60	6	Ox	batch	NH_3
Porites coral	JCp-1	24.44	0.56	6	U	column	NH_3
Porites coral	JCp-1	24.41	0.30	6	U	batch	NH_3
Porites coral	JCp-1	24.36	0.51	2	Ox	column	NH_3
Porites coral	JCp-1	24.24	0.38	2	Ox	batch	NH_3
Porites coral	NEP-1	26.56	0.34	2	U	batch	NH_3
Porites coral	NEP-1	25.51	0.38	2	Ox	column	NH_3
Porites coral	NEP-1	25.34	0.78	2	Ox	batch	NH_3
Porites coral	NEP-1	25.52	0.46	2	U	column	NH_3
Porites coral	NEP-1	25.92	0.12	2	U	batch	NH_3
Porites coral	NEP-1	25.96	0.30	2	Ox	batch	NH_3
Temperate coral	OCU-9	24.04	na	1	Ox	batch	NH_3
Temperate coral	OCU-10	23.98	na	1	Ox	batch	NH_3
Temperate coral	OCU-11	24.34	na	1	Ox	batch	NH_3
Coralline alga	JR-19	39.94	0.12	2	Ox	batch	NH_3
Coralline alga	JR-20	32.65	0.46	2	Ox	batch	NH_3
Coralline alga	JR-20	32.68	0.22	2	Ox	column	NH_3
Coralline alga	JR-21	35.07	na	1	Ox	batch	NH_3
Tropical urchin	JR-56	19.00	0.36	2	Ox	batch	NH_3
Tropical urchin	JR-57	18.64	0.11	2	Ox	batch	NH_3
Tropical urchin	JR-58	18.49	0.09	2	Ox	batch	NH_3
Temperate urchin	JR-64	14.96	0.10	2	Ox	column	NH_3
Temperate urchin	JR-64	17.60	0.80	2	Ox	batch	d-DIHEN
Temperate urchin	JR-65	17.11	1.10	2	Ox	batch	NH_3
Temperate urchin	JR-66	15.43	0.11	2	Ox	batch	NH_3
Serpulid worm tube	JR-1	19.44	na	1	Ox	batch	NH_3
Serpulid worm tube	JR-2	19.13	na	1	Ox	batch	NH_3
Serpulid worm tube	JR-3	19.21	na	1	Ox	batch	NH_3
American oyster	JR125	16.18	0.16	2	Ox	column	NH_3
American oyster	JR125	15.90	0.60	2	Ox	batch	d-DIHEN
American oyster	JR125	16.00	0.32	2	U	batch	NH_3

Table 4. Summary of the average and standard deviation (SD) of $\delta^{11}B$ 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 a;., 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~~analysed. ‘NA’ = not available, only one biological replicate analysed. **Note:** SD is calculated from measurements of different individuals of the same species, thereby ~~and this~~ reflecting interspecimen variability. Variability arising from intra-specimen variation (~~reflecting~~ i.e., variability within a single specimen) and analytical error is provided in Table 3.

Sample Type	**Scientific Name**	**$\delta^{11}B$ (SD)**	**pH_{CF}**	**pH_{SW}**	**ΔpH**	**OA Response**	**Mineralogy**
Coralline alga	*Neogoniolithion* sp.	**35.89** (3.71)	9.4	8.1	1.3	Parabolic	HMC
Temperate coral	*Oculina arbuscula*	**24.12** (0.19)	8.5	8.1	0.4	Threshold	Aragonite
Tropical urchin	*Eucidaris tribuloides*	**18.71** (0.26)	8.1	8.0	0.1	Threshold	HMC
Serpulid worm	*Hydroides crucigera*	**19.26** (0.16)	8.2	8.1	0.1	Negative	Aragonite+HMC
Temperate urchin	*Arbacia punctulata*	**16.28** (0.86)	7.9	8.0	-0.1	Parabolic	HMC
American oyster	*Crassostrea virginica*	**16.03** (NA)	7.9	8.2	-0.3	Negative	LMC

Table 5. Previously published δ^{11}B analyses of biogenic marine carbonates and seawater samples.

Sample	**Mineralogy**	**δ^{11}B range (‰)**	**Reference**
Modern Coral	Aragonite	26.7-31.9	(Vengosh et al., 1991)
Modern Coral	Aragonite	23.0-24.7	(Hemming and Hanson, 1992)
Modern Coral	Aragonite	23.5-27.0	(Gaillardet and Allègre, 1995)
Modern Coral	Aragonite	23.9-26.2	(Hemming et al., 1998)
Modern Coral	Aragonite	25.2	(Allison and Finch, 2010)
Modern Coral	Aragonite	23.56-27.88	(Anagnostou et al., 2012)
Modern Coral	Aragonite	21.5-28.0	(Dishon et al., 2015)
Modern Coral	Aragonite	21.76-23.19	(Dissard et al., 2012)
Deep Sea Coral	Calcitic	13.7-17.3	(Farmer et al., 2015)
Modern Coral	Aragonite	18.52-23.96	(Holcomb et al., 2014)
Modern Coral	Aragonite	21.1-24.9	(Hönisch et al., 2004)
Modern Coral	Aragonite	23.2-28.7	(McCulloch et al., 2012)
Deep Sea Coral	Calcitic	15.5	(McCulloch et al., 2012)
Modern Coral	Aragonite	22.5-24.0	(Reynaud et al., 2004)
Modern Coral	Aragonite	31.1-35.7	(Rollion-Bard et al., 2011a)
Modern Coral	Aragonite	18.6-30.6	(Rollion-Bard et al., 2011b)
Modern Coral	Aragonite	21-24.5	(Schoepf et al., 2014)
Modern Coral	Aragonite	23.6-25.2	(D'Olivo et al., 2015)
Ancient Coral	Aragonite	23.6-27.1	(Douville et al., 2010)
Ancient Coral	Aragonite	24.5-27.1	(Kubota et al., 2014)
Ancient Coral	Aragonite	22.5-25.5	(Liu et al., 2009)
Modern Coral	Aragonite	21.1-25.4	(Wei et al., 2009)
Planktonic Foraminifera	Calcite	14.2-19.8	(Vengosh et al., 1991)
Planktonic Foraminifera	Calcite	22.0-23.3	(Sanyal et al., 1995)
Planktonic Foraminifera	Calcite	18.4	(Sanyal et al., 1997)
Benthic Foraminifera	Calcite	13.3, 20.3, 32.0	(Vengosh et al., 1991)
Benthic Foraminifera	Calcite	20.5, 21.4	(Sanyal et al., 1995)

Bulk Foraminifera	Calcite	10.5, 11.5, 14.8, 16.2, 17.0	(Spivak et al., 1993)
Planktonic Foraminifera	Calcite	17.1, 22.9	(Kasemann et al., 2009)
Planktonic Foraminifera	Calcite	20.6-25.4	(Ni et al., 2007)
Benthic Foraminifera	Calcite	14.5-16.8	(Rae et al., 2011)
Benthic Foraminifera	Calcite	18-30.1	(Rollion-Bard and Erez, 2010)
Benthic Foraminifera	Calcite	15.8-17.4	(Yu et al., 2010)
Planktonic Foraminifera	Calcite	16.9-17.9	(Yu et al., 2013)
Planktonic Foraminifera	Calcite	19.1-22.2	(Bartoli et al., 2011)
Planktonic Foraminifera	Calcite	16.2-19.8	(Foster, 2008)
Planktonic Foraminifera	Calcite	15.2-17.2	(Foster et al., 2012)
Benthic Foraminifera	Calcite	13.09-13.37	(Foster et al., 2012))
Planktonic Foraminifera	Calcite	18.9-21.8	(Foster and Sexton, 2014)
Planktonic Foraminifera	Calcite	20.8-23.3	(Hönisch and Hemming, 2005)
Planktonic Foraminifera	Calcite	21.7-23.4	(Hönisch et al., 2009)
Benthic Foraminifera	Calcite	18.0	(Kaczmarek et al., 2015)
Planktonic Foraminifera	Calcite	15.1-16.4, 18.9-21.4	(Martínez-Botí et al., 2015a)
Planktonic Foraminifera	Calcite	19.1-19.8, 19.4-20.8	(Martínez-Botí et al., 2015b)
Planktonic Foraminifera	Calcite	24.2-25.7	(Palmer et al., 2010)
Mixed Foraminifera	Calcite	19.4-27.7	(Palmer, 1998)
Mixed Foraminifera	Calcite	20.8-26.6	(Pearson and Palmer, 1999)
Planktonic Foraminifera	Calcite	11-13.5*, 21.6-25.5	(Pearson and Palmer, 2000)
Benthic Foraminifera	Calcite	15.2-16.2	(Rae et al., 2014)
Planktonic Foraminifera	Calcite	13.6-15.8	(Penman and Hönisch, 2014)
Echinoid	High-Mg Calcite	22.7-22.9	(Hemming and Hanson, 1992)
Goniolithon	High-Mg Calcite	22.4	(Hemming and Hanson, 1992)
Encrusting Red Algae	High-Mg Calcite	23.0	(Hemming and Hanson, 1992)
Thecidellina	Calcite	21.5-22.5	(Hemming and Hanson, 1992)
Other Carbonates	Aragonite	19.1-24.8	(Hemming and Hanson, 1992)
Seawater	Seawater	39.9-40.2	(Hemming and Hanson, 1992)
Seawater	Seawater	37.7-40.4	(Foster et al., 2010)

Figures

Fig. 1. (a) Speciation of dissolved inorganic boron [~~(~~$B(OH)_3$ and $B(OH)_4^-$]~~)~~ as a function of seawater pH. (b) $\delta^{11}B$ of dissolved inorganic boron species as a function of seawater pH. The pK_B~~b~~ is 8.6 at 25 °C and 35 psu (Dickon, 1990), α is 1.0272 (Klochko et al., 2006), and $\delta^{11}B_{SW}$ is 39.61 (Foster et al., 2010).

Fig. 2. Examples of previously published $\delta^{11}B_{CaCO3}$-pH_{SW} trends for corals, foraminifera and brachiopods. Although many B isotope data sets are available, only studies with ≥ 3 $\delta^{11}B_{CaCO3}$-pH_{SW} data spanning a sufficiently wide range of pH_{SW} conditions were selected to illustrate the range of $\delta^{11}B_{CaCO3}$-pH_{SW} trends published to date. The two ~~gray~~grey lines ~~indicate~~ correspond to the theoretical seawater borate $\delta^{11}B$-pH curves that have been applied most frequently to interpret $\delta^{11}B$ variability in marine calcifiers. The pK_B is 8.6 at 25 °C and 35 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~~used in the ion exchange column.

Fig. 4. Boron isotopic composition (± SD) of different species of marine calcifiers~~ying organisms~~ as a function of seawater pH (± SD). The six species shown in this figure were grown under controlled pCO_2 conditions of *ca.* 409 ppm. ~~Gray~~Grey lines are theoretical seawater $\delta^{11}B_{B(OH)4-}$-pH curves based on different α that have been used to describe boron isotope fractionation between borate ion and boric acid in seawater (using pK_B of 8.6152 at 25°C and 32 psu). Although $\alpha = 1.0272$ (Klochko et al., 2006) is presently the most commonly used α, $\delta^{11}B_{B(OH)4-}$-pH curves calculated from other values of α are also shown for ~~reference~~comparison.

Fig. 5. Exploring the potential influence of pH and boron speciation on ~~carbonate~~ $\delta^{11}B_{CaCO3}$ (adapted from Rollion-Bard et al., 2011b). The solid and dashed curves represent the $\delta^{11}B_{CaCO3}$ ~~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_{CF} based on the assumptions that 0% $B(OH)_3$ is incorporated into the temperate coral skeleton, and that 0%, 30% and 75% $B(OH)_3$ is

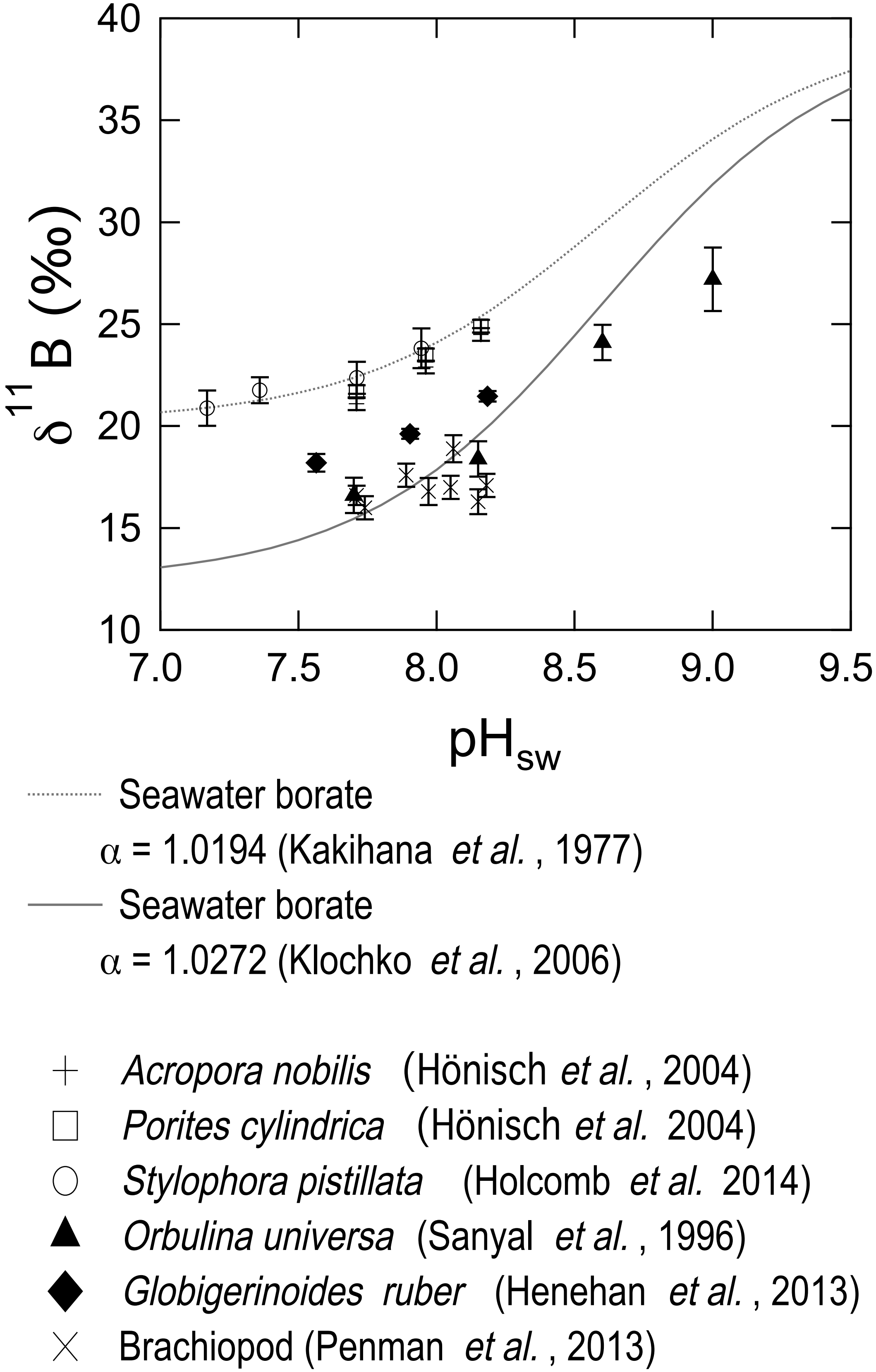
incorporated into the coralline algal skeleton. Of ~~the calcite~~all species examined, only the co~~r~~ralline algae has a $\delta^{11}B_{CaCO3}$ composition that could conceivably originate at least in part from $B(OH)_3$ incorporation, although this would require a much higher level (*ca.* 3-fold) of skeletal $B(OH)_3$ incorporation than has been previously observed (e.g., Cusack et al., 2015).

Author Contribution

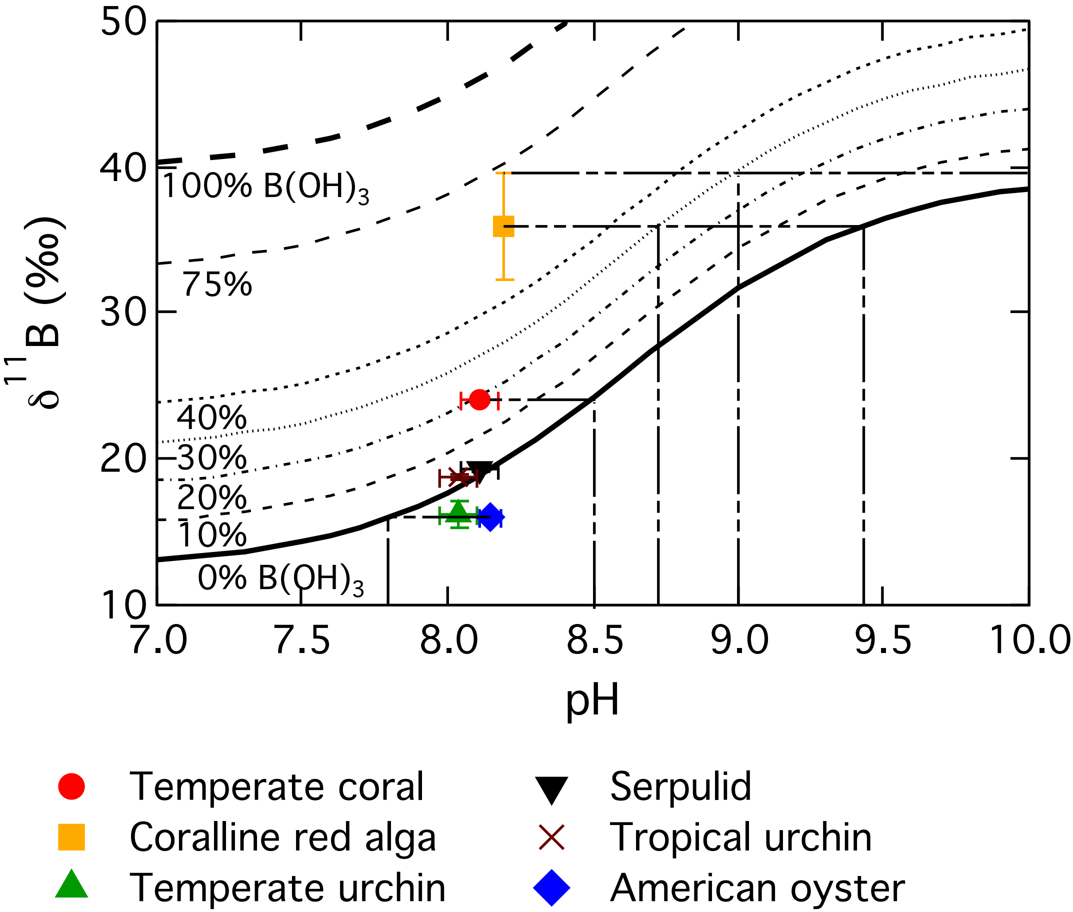
RAE and JBR conceived of the project and wrote the proposals that funded the work. JBR performed the culturing~~ed~~ ~~the organisms~~experiments. RAE, JNS, and JBR contributed to experimental design. JNS, Y-WL, MG, EP, and RAE contributed to developing the method of boron isotope analysis~~development~~. 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. This is publication #361 from Northeastern's Marine Science Center.

Acknowledgements

This work was supported by the "Laboratoire d'Excellence" LabexMER (ANR-10-LABX-19) and co-funded by a grant from the French government under the program "Investissements d'Avenir", and by a grant from the Regional Council of Brittany (SAD programme). RAE and JBR also acknowledge support from National Science Foundation grants OCE-1437166 and OCE-1437371. We thank J.-P. D'Olivo and the members of the UWA lab for supplying us with an aliquot of the NEP standard.



δ^{11}B (‰)
40
35
30
25
20
15
10
7.0
7.5
8.0
8.5
9.0
9.5
pH_{sw}
⋯ Seawater borate
α = 1.0194 (Kakihana *et al.*, 1977)
— Seawater borate
α = 1.0272 (Klochko *et al.*, 2006)
\+ *Acropora nobilis* (Hönisch *et al.*, 2004)
□ *Porites cylindrica* (Hönisch *et al.* 2004)
○ *Stylophora pistillata* (Holcomb *et al.* 2014)
▲ *Orbulina universa* (Sanyal *et al.*, 1996)
◆ *Globigerinoides ruber* (Henehan *et al.*, 2013)
× Brachiopod (Penman *et al.*, 2013)



50
40
30
20
10
7.0
7.5
8.0
8.5
9.0
9.5
10.0
pH
δ^{11}B (‰)
100% $B(OH)_3$
75%
40%
30%
20%
10%
0% $B(OH)_3$
Temperate coral
Coralline red alga
Temperate urchin
Serpulid
Tropical urchin
American oyster