δ¹¹B as monitor of calcification site pH in divergent marine calcifying organisms

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Abstract. The boron isotope composition (δ¹¹B) 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 to derive seawater pH from δ¹¹B from biogenic carbonates. It is also probable that δ¹¹B of biogenic carbonate reflects seawater pH at the organism’s site of calcification, which may or may not reflect seawater pH. Here, we report the development of methodology for measuring the δ¹¹B of biogenic carbonate samples at the multi-collector inductively coupled mass spectrometry facility at Ifremer (Plouzané, France) and the evaluation of δ¹¹B_CaCO₃ in a diverse range of marine calcifying organisms reared for 60 days in isothermal seawater (25°C) equilibrated with an atmospheric pCO₂ of ca. 409 µatm. Average δ¹¹B_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 ‰). These results are discussed in the context of each species’ proposed mechanism of biocalcification and other factors that could influence skeletal and shell δ¹¹B, 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 δ¹¹B_CaCO₃ (ca. 20 ‰) and significant discrepancies between measured δ¹¹B_CaCO₃ and δ¹¹B_CaCO₃ expected from established relationships between abiogenic δ¹¹B_CaCO₃ and seawater pH arise primarily from fundamental differences in calcifying site pH amongst the different species. These results highlight the potential utility of δ¹¹B as proxy of calcifying site pH for a wide range of calcifying taxa and underscore the importance of using species-specific seawater pH-δ¹¹B_CaCO₃ calibrations when reconstructing seawater pH from δ¹¹B of biogenic carbonates.

1 Introduction

The ability to monitor historical changes in seawater pH on both short and long timescales is necessary to understand the influence that changes in the partial pressure of atmospheric CO₂ (pCO₂) have had on the carbonate chemistry of seawater. The recent anthropogenic increase in pCO₂ 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; Gonzalez-Davlia et al., 2010; IPCC, 2014), which affects the ability of marine calcifying organisms to produce their shells and skeletons (CaCO₃; IPCC, 2014). Experimental studies have revealed that organismal responses to ocean acidification vary widely amongst taxa, highlighting the complexity of
biological responses to global change stressors (e.g., Kroeker et al., 2010; Kroeker et al., 2013; Ries et al., 2009) and necessitating a more thorough understanding of how an organism’s mechanism of biocalcification governs its specific response to ocean acidification.

1.1 Theoretical model of $\delta^{11}$B variation with pH

The boron isotope composition ($\delta^{11}$B) of biogenic CaCO$_3$ ($\delta^{11}$B$_{CaCO_3}$) has been primarily used as a paleoceanographic 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 conservatively distributed throughout the ocean (Foster et al., 2010) - making $\delta^{11}$B a potentially attractive proxy for paleo-seawater pH.

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 pH (Fig. 1) pursuant to the following equilibrium reaction:

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

In modern seawater, B(OH)$_4^-$ represents ca. 24.15% of dissolved boron, assuming that the dissociation constant (pK$_B$) between the 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. B(OH)$_3$ is enriched in $^{11}$B relative to B(OH)$_4^-$ due to 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:

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

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

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

$$\alpha \equiv \frac{\text{Boric acid}}{\text{Borate ion}}$$

A range of theoretical and empirical values for $\alpha$ has been suggested (Byrne et al., 2006; Kakhiana et al., 1977; Klochko et al., 2006; Nir et al., 2015; Palmer et al., 1987). For example, $\alpha$ of 1.0194 was calculated from theory by Kakhiana et al. (1977) and was widely applied in reconstructions of paleo-seawater pH (Hönisch et al., 2004; Kakhiana et al., 1977; Sanyal et al., 1995). Zeebe (2005) used analytical techniques and ab initio molecular orbital theory to calculate $\alpha$ ranging from 1.020 to 1.050 at 300 K. Zeebe (2005) provided several arguments in support of $\alpha \geq 1.030$, ultimately concluding that experimental work was required to determine the $\alpha$ for 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 Kakhiana et al.’s (1977) theoretical calculation of $\alpha$ (Klochko et al., 2006; Rustad and Bylaska, 2007). An empirical $\alpha$ of 1.0272 (Klochko et al., 2006), using a corrected borate vibrational spectrum term, is now considered to best describe the boron isotope fractionation between dissolved boric acid and borate ion in seawater (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, paleo-seawater pH may not simply be reconstructed by
projecting measured $\delta^{11}$B of calcium carbonate ($\delta^{11}$B$_{CaCO_3}$) onto a theoretical seawater borate $\delta^{11}$B ($\delta^{11}$B$_{B(OH)4}$)-pH curve (see also Anagnostou et al., 2012; Honisch 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$_{CaCO_3}$.

The $\delta^{11}$B-based paleo-seawater pH proxy is based on a theoretical model of $\delta^{11}$B$_{B(OH)4}$ variation with pH described by the following equation (Zeebe and Wolf-Gladrow, 2001):

$$pH = pK_B - \log \left( \frac{\delta^{11}B_{CaCO_3} - \delta^{11}B_{SW}}{(\delta^{11}B_{SW} - \alpha_B) \times (\delta^{11}B_{CaCO_3} - \epsilon_B)} \right)$$

This theoretical model of $\delta^{11}$B$_{B(OH)4}$ variation as a function of seawater pH requires knowledge of the $\alpha$ 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$_{CaCO_3}$ reflects seawater $\delta^{11}$B$_{B(OH)4}$, and thus, seawater pH (Hemming and Hanson, 1992). Although early studies assumed that $\delta^{11}$B$_{CaCO_3}$ 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$_{CaCO_3}$-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$_{CaCO_3}$ using the theoretical $\delta^{11}$B$_{B(OH)4}$-pH relationship (i.e., $\delta^{11}$B$_{CaCO_3}$-pH relationships must be empirically calibrated for the species hosting the paleo-pH proxy).

The $\delta^{11}$B-based paleo-seawater pH proxy also relies on the assumption that $\text{B(OH)}_4^-$ is the dominant species of dissolved inorganic boron incorporated into CaCO$_3$ minerals precipitated from seawater. It is well established that $\delta^{11}$B of dissolved $\text{B(OH)}_4^-$ is controlled by solution pH (c.f. Hemming and Hönisch, 2007; see discussion above). Therefore, $\delta^{11}$B$_{CaCO_3}$ should reflect pH of the precipitating solution if $\text{B(OH)}_4^-$ is indeed the dominant species of dissolved inorganic boron incorporated into CaCO$_3$, which is consistent with numerous empirical studies (see Hemming and Hönisch, 2007, for summary).

More recently, however, alternative models of boron incorporation into CaCO$_3$ have been proposed (Balant et al., 2016; Klochko et al., 2009; Noireaux et al., 2015; Uchikawa et al., 2015). These alternative models present a potential challenge to the utility of boron isotopes in reconstructing calcifying fluid and paleo-seawater pH (Balant et al., 2016; Klochko et al., 2009; Mavromatis et al., 2015; Noireaux et al., 2015; Uchikawa et al., 2015). These studies present evidence consistent with the incorporation of boric acid, along with borate, into some carbonates (e.g. Noireaux et al., 2015; Uchikawa et al., 2015) and/or the 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 suggest that calcite is more prone to boric acid incorporation than aragonite (e.g. Noireaux et al., 2015). However, these studies evaluated inorganic carbonates precipitates from fluids of compositions that differed substantially from seawater; it is yet to be determined whether boric acid incorporation is equally as prevalent in carbonates that are precipitated from seawater. Nevertheless, we evaluate boric acid incorporation as an alternative to our hypothesis that calcifying fluid pH exerts primary control over the $\delta^{11}$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 (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:

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

which are respectively governed by the following stoichiometric dissociation constants:

\[
K^*_1 = [\text{HCO}_3^-][\text{H}^+] / [\text{H}_2\text{CO}_3]
\]

and

\[
K^*_2 = [\text{CO}_3^{2-}][\text{H}^+] / [\text{HCO}_3^-]
\]

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

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

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

The decrease in pH$_{\text{SW}}$ that will accompany the rise in anthropogenic atmospheric $\rho$CO$_2$ 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 $\rho$CO$_2$ increases. Organisms able to strongly regulate pH of their calcifying fluid (pH$_{\text{CF}}$), despite reduced external pH, should convert much of this increased DIC, occurring primarily as HCO$_3^-$, back into the CO$_3^{2-}$ needed for calcification (Ries, 2011b, 2011a; Ries et al., 2009). Thus, an organism’s specific response to CO$_2$-induced ocean acidification should be critically dependent upon that organisms’ ability to regulate pH at their site of calcification.

Marine calcifiers biomineralize in diverse ways, with some calcifiers’ mechanisms of biomineralization 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; 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., Marie et al., 2012; Mount et al., 2004; Weiner et al., 1984). Coralline red algae are also thought to precipitate primarily high-Mg calcite extracellularly, but within a chemically controlled fluid bound by adjacent cells. 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\(_{\text{CF}}\), 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\(_{\text{CF}}\) 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 \(\delta^{11}\text{B}_{\text{CaCO}_3}\)

Numerous studies have documented systematic relationships between pH\(_{\text{SW}}\) and the \(\delta^{11}\text{B}_{\text{CaCO}_3}\) composition of foraminiferal shells and coral skeletons (Fig. 2) that are generally consistent with theoretically derived relationships between seawater pH and \(\delta^{11}\text{B}_{\text{B(OH)}_4^-}\). However, the observed relationships between biogenic \(\delta^{11}\text{B}_{\text{CaCO}_3}\) and pH\(_{\text{SW}}\) vary widely amongst taxa (Fig. 2), and are generally 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).

One hypothesis for the discrepancies between the expected \(\delta^{11}\text{B}_{\text{CaCO}_3}\)-pH relationship and those 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\(_{\text{CF}}\)) that is substantially elevated relative to that of their external seawater (pH\(_{\text{SW}}\)). For example, prior studies have shown that for a given pH\(_{\text{SW}}\), \(\delta^{11}\text{B}_{\text{CaCO}_3}\) of the coral species Porites cylindrica and Acropora nobilis are moderately elevated relative to \(\delta^{11}\text{B}_{\text{CaCO}_3}\) of the foraminifera Globigerinoides sacculifer and substantially elevated relative to the 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\(_{\text{CF}}\) of mussels. This is consistent with pH microelectrode (Al-Horani et al., 2003; Ries, 2011a), and fluorescent pH dye data (Venn et al., 2009, 2011, 2013), suggesting that scleractinian corals elevate their pH\(_{\text{CF}}\) to 8.5 – 10, versus their external pH\(_{\text{SW}}\) of 8, that foraminifera maintain their pH\(_{\text{CF}}\) between 8 and 9 (Jorgensen et al., 1985; Rink et al., 1998), and that bivalves maintain their pH\(_{\text{CF}}\) between 7.5 and 8 (Crenshaw, 1972).

Here, we investigate differences in \(\delta^{11}\text{B}_{\text{CaCO}_3}\)-pH relationships amongst taxonomically diverse biogenic calcification systems and discuss the compatibility of these observations with the hypothesis that \(\delta^{11}\text{B}_{\text{CaCO}_3}\) of biogenic carbonate is recording pH\(_{\text{CF}}\), rather than pH\(_{\text{SW}}\)—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 \(\delta^{11}\text{B}_{\text{CaCO}_3}\) composition of a taxonomically broad range of taxa, each employing different mechanisms of calcification yet all cultured under equivalent laboratory conditions (Ries et al., 2009), we are able to empirically assess biological controls on the \(\delta^{11}\text{B}_{\text{CaCO}_3}\) 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 \(^{11}\text{B}/^{10}\text{B}\) 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, removing caps of sample vials 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 was a Millipore Direct-Q water purification system with a specific resistivity of 18.2 MΩ·cm. All HNO₃ solutions are obtained from dilutions using Aristar Ultra-high purity acid. The 0.5 N NH₄OH solutions are boron-cleaned by exchange with boron-specific resin (Amberlite IRA 743). UHQ water is buffered to pH 7 with the boron-cleaned NH₄OH. 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

This study evaluated the δ¹¹B CaCO₃ of 6 divergent species of marine calcifiers reared for 60 days in isothermal (25°C) and isosaline (32 practical salinity units; psu) seawater equilibrated with atmospheric pCO₂ 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 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 specimens per species analyzed for δ¹¹B CaCO₃.

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 δ¹¹B 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 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).

Two methods of B extraction are presented: batch and column chemistry. 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 four biogenic CaCO₃ 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 NH₄OH) H₂O₂ 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 HNO₃ 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 % NH₄OH), centrifuged, and the water was removed. Dissolution of each sample was then performed by addition of 20 µL of 3 M HNO₃ followed by 300 µL of 0.05 M HNO₃. The pH of each sample was then adjusted to pH 7 with 0.2 M NH₄OH, following partition coefficients for the B-specific resin reported by Lemarchand et al. (2002). For both the batch and the column chemistry methods, the resin is pre-cleaned and conditioned to pH 7 prior to sample loading.

2.4.2 Column chemistry method

A column chemistry protocol for B extraction (described in Table 1) was developed based on methods described by Wang et al. (2010) and Foster et al. (2013). Briefly, the columns were washed with pH-buffered UHQ-H₂O (pH=7), 0.5 M HNO₃, and again with pH-buffered UHQ-H₂O. After confirming that the eluent was at pH 7, the sample was loaded onto the resin and washed multiple times (1500 µL x 3) with pH-buffered UHQ in order to remove any cations, after which the B was eluted in 1000 µL of 0.5 M HNO₃. Column yields were greater than 95 % (Fig. 3) and elution tails of every sample were checked with an additional 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 analysis by MC-ICPMS to verify 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 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 HNO₃, and then rinsed three times with 500 µL of UHQ water 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 HNO₃ and shaken again for 15 min to promote anion exchange between the resin and solution. A final tail-check was performed with 100 µL of 0.05 M HNO₃ to ensure that all of the B was recovered in the initial 500 µL 0.05 M HNO₃ solution.

2.5 Procedural blanks

The total yield of B from procedural blanks, which should reflect reagent, air-borne and procedural contamination, was sub-nanogram (lowest yields for column and batch methods were 0.5 ng and 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 δ¹¹B by MC-ICPMS is the elimination of residual boron from prior analyses (i.e., ‘memory effects’). In order to evaluate memory effects, multiple concentrations (30 ppb to 130 ppb) of a standard solution (NIST SRM 951) were analysed. After washing out the MC-ICPMS with a solution of 0.05 M HNO₃ for several minutes, the residual ¹¹B and ¹⁰B signals were in the range of 10 – 80 mV, equivalent to 5 % (30 ppb) and 3 % (130 ppb), respectively (see
Fig. S1 for $^{11}$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. The boron isotope ratios are reported 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 is commonly used 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$_{CaCO_3}$-based estimates of pH$_{CF}$ to the analytical uncertainty cited above, two different injection methods (described below) were evaluated to determine 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 UHQ-H$_2$O 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$_{NH_3}$ = 24.45 ± 0.28 ‰, $\delta^{11}$B$_{d-DIHEN}$ = 24.30 ± 0.16 ‰).
and a giant clam (Tridacna gigas; δ\(^{11}\)B\(_{\text{NH4}}\) = 16.65 ± 0.39 ‰, δ\(^{11}\)B\(_{\text{d-DIHEN}}\) = 17.5 ± 0.69 ‰), and their values established via inter-laboratory calibration (δ\(^{11}\)B = 24.36 ± 0.51 ‰, n = 10 and 16.34 ± 0.64 ‰, respectively; Gutjahr et al. 2014; see Table 3). In addition, both column and batch methods were evaluated using the NEP laboratory standard (Porites sp.), a temperate urchin, a hard clam, and an oyster. As shown in Table 3, good agreement was achieved between δ\(^{11}\)B\(_{\text{CaCO3}}\) 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 δ\(^{11}\)B\(_{\text{CaCO3}}\) 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 δ\(^{11}\)B\(_{\text{CaCO3}}\), 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 temperate oyster C. virginica (16.03 ‰; n = 1). Therefore, a range of ca. 20 ‰ in δ\(^{11}\)B\(_{\text{CaCO3}}\) was observed across all species evaluated in this study (Table 3 and 4). Notably, these are the first published δ\(^{11}\)B\(_{\text{CaCO3}}\) data for serpulid worm tubes and oysters.

3.3 Compatibility of the interspecific range of δ\(^{11}\)B\(_{\text{CaCO3}}\) with established seawater borate δ\(^{11}\)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\(_{\text{SW}}\) could not have been a significant driver of the observed interspecific variability in δ\(^{11}\)B\(_{\text{CaCO3}}\) (ca. 20 ‰; Tables 3 and 4). In order to evaluate this ca. 20 ‰ interspecific variability in δ\(^{11}\)B, the data are plotted against measured pH\(_{\text{SW}}\) and graphically compared with theoretical borate δ\(^{11}\)B-pH curves often used to interpret δ\(^{11}\)B\(_{\text{CaCO3}}\) data in the context of pH\(_{\text{SW}}\) (Fig. 4). Clear offsets from the seawater borate δ\(^{11}\)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 δ\(^{11}\)B-pH curve is discussed below.

4 Discussion

4.1 Appropriateness of method for analysing δ\(^{11}\)B\(_{\text{CaCO3}}\) 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 δ\(^{11}\)B\(_{\text{CaCO3}}\) 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. 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. 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 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 contamination) used in the separation process.

4.2 The δ\(^{11}\)B\(_{\text{CaCO}_3}\) compositions of a diverse range of marine calcifiers

The six species investigated exhibited a broad spectrum of δ\(^{11}\)B\(_{\text{CaCO}_3}\) compositions, ranging from 16.03 ‰ to 35.89 ‰ (Table 4) despite exposure of all species to approximately equivalent pH\(_{SW}\) of 8 (see Table 4). We cannot constrain whether the relationship between δ\(^{11}\)B\(_{\text{CaCO}_3}\) and δ\(^{11}\)B of borate significantly differs from unity in this experiment with a single pH\(_{SW}\). Because δ\(^{11}\)B\(_{\text{calcifying fluid}}\) at the species’ sites of calcification cannot be measured or calculated from the data at hand, it cannot be directly compared with the measured δ\(^{11}\)B\(_{\text{CaCO}_3}\) to determine if δ\(^{11}\)B\(_{\text{calcifying fluid}}\) necessarily reflects calcifying fluid δ\(^{11}\)B\(_{\text{calcifying fluid}}\) and, thus, pH\(_{CF}\). Assuming that only the borate ion is incorporated into biogenic CaCO\(_3\) (i.e., δ\(^{11}\)B\(_{\text{CaCO}_3} = \text{calcifying fluid} \ \delta^{11}\text{B}_{\text{biogenic}}\)), the wide variation in δ\(^{11}\)B\(_{\text{CaCO}_3}\) (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 δ\(^{11}\)B\(_{\text{CaCO}_3}\) amongst the species (16.03 ‰ to 35.89 ‰) translates to an approximate range in pH\(_{CF}\) of 7.9 – 9.4.

The amount of boron (i.e., B/Ca) co-precipitated 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 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). 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. 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 δ\(^{11}\)B\(_{\text{CaCO}_3}\) composition than calcite-producing species (urchins, coralline algae, oysters) if shell mineralogy was the primary driver of the observed interspecific variation in δ\(^{11}\)B\(_{\text{CaCO}_3}\) compositions – a trend that is not observed (Fig. 4). Thus, interspecific differences in polymorph mineralogy cannot, alone, explain the species’ disparate δ\(^{11}\)B\(_{\text{CaCO}_3}\) compositions. The more parsimonious explanation for these observed differences in δ\(^{11}\)B\(_{\text{CaCO}_3}\) 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 δ\(^{11}\)B\(_{\text{CaCO}_3}\) that differs from δ\(^{11}\)B\(_{\text{calcifying fluid}}\) of their surrounding seawater (Figs. 3 and 5). When interpreted in the context of the framework that skeletal δ\(^{11}\)B reflects pH\(_{CF}\) rather than the organism’s ambient pH\(_{SW}\), these results suggest that marine calcifiers are precipitating their CaCO\(_3\) 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 pH\(_{CF}\) exerts some control over δ\(^{11}\)B\(_{\text{calcifying fluid}}\) at the site of calcification and, hence, δ\(^{11}\)B\(_{\text{CaCO}_3}\) but that there are other species-specific effects that also influence δ\(^{11}\)B\(_{\text{CaCO}_3}\) composition. The compatibility of these two hypotheses with existing models of biomineralization and observed δ\(^{11}\)B\(_{\text{CaCO}_3}\) for the various marine calcifiers investigated in the present study are discussed below.

4.2.1 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 δ¹¹B\textsubscript{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 δ¹¹B\textsubscript{CaCO₃} composition of any other calcifying marine organism evaluated to date (Table 5). Of particular interest, one of the coralline red algae specimens evaluated in this study exhibited δ¹¹B\textsubscript{CaCO₃} (39.94 ‰, Table 3) similar to the average δ¹¹B of the total dissolved boron in seawater (i.e., comprising the δ¹¹B 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\textsubscript{SW} of 8.1, the δ¹¹B\textsubscript{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, which is not consistent with prior observations for inorganic and biogenic calcite. For example, Cusack et al., 2015, reported 30% trigonal boron in the calcite lattice of a different species of coralline alga. Therefore, boric acid incorporation alone cannot explain the anomalously elevated δ¹¹B\textsubscript{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 the B in the calcite lattice of coralline algal skeleton is indeed incorporated directly as trigonal boron, as reported by Cusack et al. (2015), pH\textsubscript{CF} would still need to be as high as 9 to explain the anomalously high δ¹¹B\textsubscript{CaCO₃} (see Fig. 5). Short et al. (2015) observed that epiphytic turf algae can increase pH\textsubscript{SW} up to 9 within their diffusive boundary layer, driven by the algae’s photosynthetic drawdown of aqueous CO₂, lending further support to the idea that other types of algae, such as coralline red algae, could maintain their calcifying fluid at or above pH 9. Thus, δ¹¹B\textsubscript{CaCO₃} compositions of coralline red algae may indeed reflect substantially elevated pH\textsubscript{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 δ¹¹B\textsubscript{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 ¹¹B when compared with a theoretical borate δ¹¹B-pH curve (see Figures 2 and 4). The main vital effect typically used to explain ¹¹B-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 δ¹¹B 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 δ¹¹B\textsubscript{CaCO₃} for the tropical urchin E. tribuloides (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) evaluated in this study, which were both reared at equivalent seawater conditions (pH\textsubscript{SW} = 8.0; 25 °C; 32 psu; Table 4), are lower than δ¹¹B\textsubscript{CaCO₃} previously reported for other echinoid species (see...
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$_{CaCO_3}$ is similar to $\delta^{11}$B of dissolved borate. The difference between the $\delta^{11}$B$_{CaCO_3}$ 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 ($\pm 0.26$‰ for the tropical urchin; $\pm 0.86$‰ for the temperate urchin, determined as standard deviation, see Table 5). However, the urchins could achieve this deviation in $\delta^{11}$B$_{CaCO_3}$ by adjusting pH of their calcifying environment by only $\pm 0.1$ unit (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$_{CaCO_3}$ from seawater borate $\delta^{11}$B indeed reflect urchins’ ability to modify pH at their site of calcification, these modifications appear to be relatively minor (i.e., $\pm 0.1$ 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. The relatively low $\delta^{11}$B of the urchins’ tests is not consistent with the hypothesis that significant boric acid is incorporated into the urchins’ high-Mg calcite lattice (Fig. 5).

### 4.2.4 Serpulid worm tube (H. crucigera)

The average $\delta^{11}$B$_{CaCO_3}$ for the calcareous tube of the serpulid worm *H. crucigera* evaluated in this study ($19.26 \pm 0.16$‰; 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* produces its calcareous tube from a combination of aragonite and high-Mg calcite (HMC; Ries, 2011b). The worm 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$_{B(OH)4}$ of 18.38‰, which is $0.88$‰ less than $\delta^{11}$B$_{CaCO_3}$ measured for this species. Similar to the tropical urchin discussed above, the serpulid worm could generate this divergence in $\delta^{11}$B$_{CaCO_3}$ from seawater $\delta^{11}$B$_{B(OH)4}$ 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$_{CaCO_3}$ for the American oyster *C. virginica* evaluated in this study (16.03‰; n = 1; Tables 3 and 4) is less than the theoretical value of seawater $\delta^{11}$B$_{B(OH)4}$ 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). The specimens of *C. virginica* evaluated in this study were grown in seawater conditions (pH$_{SW}$ = 8.2; 25°C; 32 psu; Table 4) that yield a theoretical seawater $\delta^{11}$B$_{B(OH)4}$ of 19.57‰, which is $3.54$‰ greater than $\delta^{11}$B$_{CaCO_3}$ measured for this species. The observation that oyster $\delta^{11}$B$_{CaCO_3}$ is substantially less than seawater $\delta^{11}$B$_{B(OH)4}$ 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 attributed 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 CaCO₃ 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; Weiner et al., 1984) and/or maintaining elevated levels of dissolved inorganic carbon within the EPF. The oyster could generate this negative divergence in δ¹¹BCaCO₃ from seawater borate δ¹¹B by decreasing pHᵥ 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 δ¹¹BCaCO₃ falls below the theoretical seawater δ¹¹B(BOH₄⁻)-pH curve (Klochko et al., 2009; Fig 5). The relatively low δ¹¹B of the oyster calcite is not consistent with significant boric acid incorporation into the oyster’s calcite lattice (Fig. 5). To the authors’ knowledge, these are the first B isotope analyses reported for oysters.

4.3 Estimating pHᵥ from δ¹¹BCaCO₃

The six species of calcifying marine organisms investigated in the present study exhibited average δ¹¹BCaCO₃ ranging from 16.27 ‰ to 35.09 ‰ (Table 3). Given that all six species were grown under nearly equivalent controlled laboratory conditions, the large interspecific range in δ¹¹BCaCO₃ supports the hypothesis that δ¹¹BCaCO₃ of biogenic carbonates is not simply inherited from δ¹¹B(BOH₄⁻) of the organism’s surrounding seawater (see Table 5 and references therein). Rather, we assert that this species-dependent variability in δ¹¹BCaCO₃ is driven by interspecific differences in the organisms’ pHᵥ. To explore this assertion, δ¹¹BCaCO₃ values were converted to pHᵥ from measured seawater temperature, salinity, a total dissolved boron δ¹¹B 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 direct measurements of calcifying fluid temperature, salinity, and total dissolved boron δ¹¹B, these parameters are assumed to 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), these calculations yield a pHᵥ (in order of decreasing magnitude) of 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) and American oyster (C. virginica).

4.3.1 Nonlinearity of δ¹¹BCaCO₃-pHᵥ relationship relative to pKₐ

The determination of pHᵥ from pKₐ, δ¹¹B of calcifying fluid (δ¹¹Bᵥ), and δ¹¹BCaCO₃ can be summarized with the following equation (Eq. 1):

\[
\text{pH}_v = \text{pK}_a - \log ((\delta^{11}B_{CF} - \delta^{11}B_{CaCO3})/ (\delta^{11}B_{CF} - (\alpha \times \delta^{11}B_{CaCO3}) - 1000(\alpha - 1))); \quad (1)
\]

where pKₐ is 8.6152 (at 25°C and 32 psu; Dickson, 1990), δ¹¹Bᵥ is 39.61 ‰ (inherited from δ¹¹B_sw; Foster et al., 2010), and α is 1.0272 (Klochko et al., 2006). Thus, Eq. 1 allows for the calculation of δ¹¹BCaCO₃ across a range of pHᵥ (Fig. 1b; Table S1).

The sensitivity of δ¹¹BCaCO₃ to changes in pHᵥ increases as pHᵥ approaches pKₐ (8.6152; Table S1). For example, a change in pHᵥ from 7.75 to 7.80 predicts a δ¹¹BCaCO₃ difference of 0.35 ‰ (15.77 ‰ – 15.42 ‰), whereas a change in pH from 8.35 to 8.40 predicts a δ¹¹BCaCO₃ difference of 0.74 ‰ (22.59 ‰ – 21.85 ‰). Thus, the relationship between pHᵥ and δ¹¹BCaCO₃ is nonlinear over the pHᵥ range of interest (7 < pH < 10), with pH having the greatest influence on δ¹¹BCaCO₃ as fluid pHᵥ approaches pKₐ.

Fortuitously, the calcifiers investigated in the present study maintain their pHᵥ within approximately 1 pH unit of pKₐ (i.e., over the interval where small differences in pHᵥ cause relatively large differences in δ¹¹BCaCO₃). Therefore, for these organisms, it will be easier to obtain precise measurements of expected differences in δ¹¹BCaCO₃ and, thus, differences in pHᵥ.
Conversely, it will be harder to obtain precise measurements of the differences in $\delta^{11}$B$_{\text{CaCO}_3}$ (and pH$_{\text{CF}}$) for calcifiers that maintain their pH$_{\text{CF}}$ more distal from pK$_B$—if such calcifiers indeed exist.

Along these same lines, slight differences in pH$_{\text{SW}}$ of the experimental treatments (also proximal to pK$_B$) could conceivably translate to relatively large changes in $\delta^{11}$B$_{\text{B(OH)}_4}$ amongst the species’ seawater treatments and, thus, their calcifying fluid $\delta^{11}$B$_{\text{B(OH)}_4}$ and $\delta^{11}$B$_{\text{CaCO}_3}$. However, the small range of pH$_{\text{SW}}$ for the different species’ experimental treatments (8.0-8.2; Table 4) could only account for a 2.24‰ range in $\delta^{11}$B$_{\text{CaCO}_3}$ (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$_{\text{CaCO}_3}$ (ca. 20‰) observed for the investigated species requires an alternative explanation, such as fundamental differences in their pH$_{\text{CF}}$.

4.3.2 Sensitivity of $\delta^{11}$B-derived pH$_{\text{CF}}$ to choice of $\alpha$

As discussed in the Introduction (section 1.1), much work has gone into establishing an $\alpha$ 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 paleo-pH reconstructions using a theoretical value of 1.0194 (Kakihana et al., 1977; see Fig. 2). An empirical $\alpha$ of 1.0272 (Klochko et al., 2006) has now been shown to better predict $\delta^{11}$B$_{\text{B(OH)}_4}$, viz. $\delta^{11}$B$_{\text{CaCO}_3}$, across the range of pH relevant for seawater (Rollion-Bard and Erez, 2010; Xiao et al., 2014). However, $\delta^{11}$B$_{\text{CaCO}_3}$ of many species of calcifying marine organisms fall either above or below theoretical $\delta^{11}$B$_{\text{B(OH)}_4}$-pH$_{\text{SW}}$ curves. It has long been suggested (and shown for corals) that calcifying organisms diverge from the predicted $\delta^{11}$B$_{\text{CaCO}_3}$ due to their ability to modify pH of their calcifying environments (e.g., Anagnostou et al., 2012; Hönisch et al., 2004; Krief et al., 2010; McCulloch et al., 2012; Rae et al., 2011; Reynaud et al., 2004; Trotter et al., 2011; Wall et al., 2016). In the present study, species-specific divergences in $\delta^{11}$B$_{\text{CaCO}_3}$ from the theoretical $\delta^{11}$B$_{\text{B(OH)}_4}$-pH$_{\text{SW}}$ curves are interpreted as evidence of the differing capacities of calcifying marine species to modify pH$_{\text{CF}}$. Importantly, existing models of biomineralization for each species are generally compatible with these $\delta^{11}$B$_{\text{CaCO}_3}$-derived estimates of pH$_{\text{CF}}$ (see section 4.2).

Although an $\alpha$ of 1.0272 (Klochko et al., 2006) was used in the present study to estimate pH$_{\text{CF}}$, other theoretical values for $\alpha$, yielding slightly different $\delta^{11}$B$_{\text{B(OH)}_4}$-pH$_{\text{SW}}$ curves (e.g., Byrne et al., 2006; Palmer et al., 1987; see Fig. 4), will yield slightly different estimates of pH$_{\text{CF}}$ for each organism. For example, using $\alpha$ values of 1.033 (Palmer et al. 1987), 1.0285 (Byrne et al. 2006), 1.0272 (Klochko et al. 2006), and 1.0194 (Kakihana et al. 1977) and a $\delta^{11}$B$_{\text{CaCO}_3}$ of 24.12‰ (temperate coral; pH$_{\text{SW}}$ = 8.1) yields pH$_{\text{CF}}$ of 8.7, 8.6, 8.5, and 8.1, respectively—a range of 0.6 pH units. It should also be noted that the lower the $\delta^{11}$B$_{\text{CaCO}_3}$, the more sensitive the reconstructed pH is to choice of $\alpha$. For example, changing $\alpha$ from 1.0272 to 1.0330 will result in a 0.24 pH unit shift for $\delta^{11}$B$_{\text{CaCO}_3}$ of 20‰, but only a 0.12 and 0.08 pH unit shift for $\delta^{11}$B$_{\text{CaCO}_3}$ of 30‰ and 39.5‰, respectively (see Fig. 4). This underscores the importance of using the same $\alpha$ when comparing $\delta^{11}$B$_{\text{CaCO}_3}$-based estimate of pH$_{\text{CF}}$ amongst species.

4.3.3 Implications of $\delta^{11}$B$_{\text{CaCO}_3}$-derived estimates of pH$_{\text{CF}}$ for species-specific vulnerability to ocean acidification

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$_{\text{CF}}$ (relative to pH$_{\text{SW}}$) would cause HCO$_3^-$ (elevated due to increased pCO$_2$) to dissociate into CO$_3^{2-}$ for calcification, helping the organism to maintain an elevated $\Omega$ at its site of calcification (Ries et al., 2009). Evaluation of this hypothesis in the context of the results of the present...
study shows that, indeed, the different species’ $\delta^{11}\text{B}_{\text{CaCO}_3}$ and calculated pH$_{\text{CF}}$ 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 $\delta^{11}\text{B}_{\text{CaCO}_3}$ and, thus, pH$_{\text{CF}}$ than species exhibiting the more vulnerable ‘negative’ responses (e.g., oyster, serpulid worm) to ocean acidification (Table 4). The temperate urchin was the exception to this general trend, as it exhibited a relatively resilient parabolic response to ocean acidification yet maintained $\delta^{11}\text{B}_{\text{CaCO}_3}$ and, thus, pH$_{\text{CF}}$ close to that of pH$_{\text{SW}}$. These results support the assertion that interspecific differences in pH$_{\text{CF}}$ contribute to marine calcifiers’ differential responses to ocean acidification - highlighting the need for future queries into the mechanisms driving boron isotope fractionation, biomineralization, and vulnerability to ocean acidification of marine calcifying organisms.

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 $\delta^{11}\text{B}_{\text{CaCO}_3}$ of the evaluated samples and standards. The batch method of boron extraction is identified as preferable to the column chemistry method 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 $\delta^{11}\text{B}_{\text{CaCO}_3}$ 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 $\delta^{11}\text{B}_{\text{CaCO}_3}$, followed by the temperate coral O. arbuscula (24.12 ± 0.19 ‰; n = 3), the tube of the serpulid worm H. crucigera (19.26 ± 0.16 ‰; n = 3), the tropical urchin E. tribuloides (18.71 ± 0.26 ‰; n = 3), the temperate urchin A. punctulata (16.28 ± 0.86 ‰; n = 3), and the temperate oyster C. virginica (16.03 ‰; n = 1). The observed ca. 20 ‰ range in $\delta^{11}\text{B}_{\text{CaCO}_3}$ composition of the investigated species constitutes the largest range in biogenic $\delta^{11}\text{B}_{\text{CaCO}_3}$ reported to date.

Consideration of these extreme interspecific differences in $\delta^{11}\text{B}_{\text{CaCO}_3}$ in the context of existing models of biomineralization for the investigated species, combined with published measurements of pH$_{\text{CF}}$ 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’ $\delta^{11}\text{B}_{\text{CaCO}_3}$ and calculated pH$_{\text{CF}}$ 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$_{\text{CF}}$. These observations contribute to the growing body of work that uses $\delta^{11}\text{B}_{\text{CaCO}_3}$ 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 will respond to anthropogenic CO$_2$-induced ocean acidification.
References


Foster, G. L. and Sexton, P. F.: Enhanced carbon dioxide outgassing from the eastern equatorial Atlantic during the last glacial,


Marie, B., Joubert, C., Tayale, A., Zanella-Cleon, I., Belliard, C., Piquemal, D., Cochenne-Laurcoul, N., Marin, F., Gueguen,


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

<table>
<thead>
<tr>
<th>Step</th>
<th>mg resin</th>
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<th>125</th>
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<tr>
<td>1</td>
<td>Resin (μL)</td>
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<td>250</td>
<td>500</td>
</tr>
<tr>
<td>2</td>
<td>UHQ H2O at pH 7 (mL)</td>
<td>5</td>
<td>5</td>
<td>5</td>
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<tr>
<td>3</td>
<td>0.5 N HNO3 (mL)</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
</tr>
<tr>
<td>4</td>
<td>UHQ H2O at pH 7 (mL) x3</td>
<td>2.5</td>
<td>2.5</td>
<td>5</td>
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<tr>
<td>5</td>
<td>Check pH</td>
<td></td>
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<td>6</td>
<td>Sample Load (ng)</td>
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<td>536</td>
<td>536</td>
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<tr>
<td>7</td>
<td>UHQ H2O at pH 7 (mL) x3</td>
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<td>1</td>
<td>2</td>
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<tr>
<td>10</td>
<td>Check pH</td>
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<td>11</td>
<td>0.05N HNO3 (mL)</td>
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<td>0.5</td>
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<td>22</td>
<td>UHQ H2O at pH 7 (mL)</td>
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Table 2. Mass spectrometer operating conditions.

<table>
<thead>
<tr>
<th></th>
<th>d-DIHEN</th>
<th>Ammonia Addition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Injection System</td>
<td>Demountable Direct Injection High-efficiency Nebulizer</td>
<td>PFA teflon spray chamber with ESI PFA teflon 50 μL min⁻¹ nebuliser</td>
</tr>
<tr>
<td>Sample Gas Flow Rate</td>
<td>0.3 L min⁻¹</td>
<td>1.1 L min⁻¹</td>
</tr>
<tr>
<td>Running Concentrations</td>
<td>B = 50 ppb</td>
<td>B = 30 – 50 ppb (evaluated 30, 65, 130 ppb)</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>35 V ppm⁻¹, total B</td>
<td>20 V ppm⁻¹, total B</td>
</tr>
<tr>
<td>Blank Level</td>
<td>&lt; 0.5 % of ¹¹B signal after 30s in 2 % HNO₃, 0.1 % after 120s</td>
<td>&lt; 5 % of ¹¹B signal after 30s in 0.05% HNO₃, 3% after 120s</td>
</tr>
<tr>
<td>Resolution</td>
<td>Low</td>
<td>Low</td>
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<tr>
<td>Forward Power</td>
<td>1200 W</td>
<td>1200 W</td>
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<td>Accelerating Voltage</td>
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<td>10 kV</td>
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<td>Plasma Mode</td>
<td>Wet Plasma</td>
<td>Wet Plasma</td>
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<td>Cool Gas Flow Rate</td>
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<td>16 L min⁻¹</td>
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<td>Auxiliary Gas Flow Rate</td>
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<td>0.9 L min⁻¹</td>
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<td>Sampler Cone</td>
<td>Standard Ni cone</td>
<td>Standard Ni cone</td>
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<td>Skimmer Cone</td>
<td>X Ni cone</td>
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<td>Interferences</td>
<td>⁴⁰Ar⁺⁺⁺⁺ ²⁰Ne⁺⁺ resolved</td>
<td>⁴⁰Ar⁺⁺⁺⁺ ²⁰Ne⁺⁺ resolved</td>
</tr>
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<td>Accuracy</td>
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<td>0.2 ‰, 2sd, n = 6</td>
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<tr>
<td>Acquisition</td>
<td>30 x 4s</td>
<td>30 x 4s</td>
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<tr>
<td>Baselines</td>
<td>Counting times of 20 s</td>
<td>Counting times of 20 s</td>
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</table>
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*). Data are presented as average of $n$ analyses and the precision is reported as 2 standard deviations (‘2SD’). The cleaning protocol (Oxidised – ‘Ox’/Uncleaned – ‘U’), separation method (‘column’/’batch’), and injection method (‘NH$_3$’/’d-DIHEN’) are presented for comparison.

<table>
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<tr>
<th>Sample type</th>
<th>Name</th>
<th>$\delta^{11}$B (%)</th>
<th>(2SD)</th>
<th>$n$</th>
<th>Cleaning</th>
<th>Separation</th>
<th>Injection</th>
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<td>JCt-1</td>
<td>17.50</td>
<td>0.69</td>
<td>6</td>
<td>Ox</td>
<td>batch</td>
<td>d-DIHEN</td>
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<td>JCt-1</td>
<td>16.90</td>
<td>0.30</td>
<td>6</td>
<td>Ox</td>
<td>batch</td>
<td>NH$_3$</td>
</tr>
<tr>
<td>Giant clam</td>
<td>JCt-1</td>
<td>16.34</td>
<td>0.64</td>
<td>2</td>
<td>Ox</td>
<td>column</td>
<td>NH$_3$</td>
</tr>
<tr>
<td>Giant clam</td>
<td>JCt-1</td>
<td>16.24</td>
<td>0.42</td>
<td>2</td>
<td>U</td>
<td>batch</td>
<td>NH$_3$</td>
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<td>JCp-1</td>
<td>24.52</td>
<td>0.34</td>
<td>6</td>
<td>Ox</td>
<td>column</td>
<td>NH$_3$</td>
</tr>
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<td>JCp-1</td>
<td>24.30</td>
<td>0.16</td>
<td>10</td>
<td>Ox</td>
<td>batch</td>
<td>d-DIHEN</td>
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<td>NEP-1</td>
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<td>OCU-9</td>
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</table>
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$_{SW}$ ($\Delta$H), calcification response to ocean acidification experiments ('OA Response'; Ries et al., 2009), and shell/skeletal mineralogy ('HMC' = high-Mg calcite; 'LMC' = low-Mg calcite; Ries et al., 2009). In most cases 3 biological replicates of each species were analysed. ‘NA’ = not available, only one biological replicate analysed. Note: SD is calculated from measurements of different individuals of the same species, thereby reflecting interspecimen variability. Variability arising from intra-specimen variation (i.e., variability within a single specimen) and analytical error is provided in Table 3.

<table>
<thead>
<tr>
<th>Sample Type</th>
<th>Scientific Name</th>
<th>$\delta^{11}$B (SD)</th>
<th>pH$_{CF}$</th>
<th>pH$_{SW}$</th>
<th>$\Delta$H</th>
<th>OA Response</th>
<th>Mineralogy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coralline alga</td>
<td>Neogoniolithion sp.</td>
<td>35.89 (3.71)</td>
<td>9.4</td>
<td>8.1</td>
<td>1.3</td>
<td>Parabolic</td>
<td>HMC</td>
</tr>
<tr>
<td>Temperate coral</td>
<td>Oculina arbuscula</td>
<td>24.12 (0.19)</td>
<td>8.5</td>
<td>8.1</td>
<td>0.4</td>
<td>Threshold</td>
<td>Aragonite</td>
</tr>
<tr>
<td>Tropical urchin</td>
<td>Eucidaris tribuloides</td>
<td>18.71 (0.26)</td>
<td>8.1</td>
<td>8.0</td>
<td>0.1</td>
<td>Threshold</td>
<td>HMC</td>
</tr>
<tr>
<td>Serpulid worm</td>
<td>Hydroides crucigera</td>
<td>19.26 (0.16)</td>
<td>8.2</td>
<td>8.1</td>
<td>0.1</td>
<td>Negative</td>
<td>Aragonite+HMC</td>
</tr>
<tr>
<td>Temperate urchin</td>
<td>Arbacia punctulata</td>
<td>16.28 (0.86)</td>
<td>7.9</td>
<td>8.0</td>
<td>-0.1</td>
<td>Parabolic</td>
<td>HMC</td>
</tr>
<tr>
<td>American oyster</td>
<td>Crassostrea virginica</td>
<td>16.03 (NA)</td>
<td>7.9</td>
<td>8.2</td>
<td>-0.3</td>
<td>Negative</td>
<td>LMC</td>
</tr>
</tbody>
</table>
Table 5. Previously published $\delta^{11}$B analyses of biogenic marine carbonates and seawater samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mineralogy</th>
<th>$\delta^{11}$B range (‰)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>26.7-31.9</td>
<td>(Vengosh et al., 1991)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>23.0-24.7</td>
<td>(Hemming and Hanson, 1992)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>23.5-27.0</td>
<td>(Gaillardet and Allègre, 1995)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>23.9-26.2</td>
<td>(Hemming et al., 1998)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>25.2</td>
<td>(Allison and Finch, 2010)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>23.56-27.88</td>
<td>(Anagnostou et al., 2012)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>21.5-28.0</td>
<td>(Dishon et al., 2015)</td>
</tr>
<tr>
<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>21.76-23.19</td>
<td>(Dissard et al., 2012)</td>
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<tr>
<td>Deep Sea Coral Calcitic</td>
<td>Calcitic</td>
<td>13.7-17.3</td>
<td>(Farmer et al., 2015)</td>
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<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>18.52-23.96</td>
<td>(Holcomb et al., 2014)</td>
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<td>Aragonite</td>
<td>21.1-24.9</td>
<td>(Hönisch et al., 2004)</td>
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<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
<td>23.2-28.7</td>
<td>(McCulloch et al., 2012)</td>
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<td>Deep Sea Coral Calcitic</td>
<td>Calcitic</td>
<td>15.5</td>
<td>(McCulloch et al., 2012)</td>
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<td>(Reynaud et al., 2004)</td>
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<td>(Rollion-Bard et al., 2011a)</td>
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<td>(Rollion-Bard et al., 2011b)</td>
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<td>Modern Coral Aragonite</td>
<td>Aragonite</td>
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<td>(Schoepf et al., 2014)</td>
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<td>Bulk Foraminifera</td>
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<td>(Kasemann et al., 2009)</td>
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<td>(Ni et al., 2007)</td>
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<td>(Rae et al., 2011)</td>
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<td>(Rollion-Bard and Erez, 2010)</td>
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<td>(Yu et al., 2010)</td>
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<td>(Bartoli et al., 2011)</td>
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<td>(Foster, 2008)</td>
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<td>Calcite</td>
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<td>(Foster et al., 2012)</td>
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<td>Benthic Foraminifera</td>
<td>Calcite</td>
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<td>(Foster et al., 2012)</td>
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<td>Mixed Foraminifera</td>
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<td>(Foster and Sexton, 2014)</td>
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<td>(Hönisch and Hemming, 2005)</td>
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<td>(Hönisch et al., 2009)</td>
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<td>(Palmer, 1998)</td>
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<td>(Rae et al., 2014)</td>
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<td>(Penman and Hönisch, 2014)</td>
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<td>(Hemming and Hanson, 1992)</td>
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<td>Goniolithon</td>
<td>High-Mg Calcite</td>
<td>22.4</td>
<td>(Hemming and Hanson, 1992)</td>
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<td>Encrusting Red Algae</td>
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<td>(Hemming and Hanson, 1992)</td>
</tr>
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<td>Thecidellina</td>
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<td>21.5-22.5</td>
<td>(Hemming and Hanson, 1992)</td>
</tr>
<tr>
<td>Other Carbonates</td>
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<td>19.1-24.8</td>
<td>(Hemming and Hanson, 1992)</td>
</tr>
<tr>
<td>Seawater</td>
<td>Seawater</td>
<td>39.9-40.2</td>
<td>(Hemming and Hanson, 1992)</td>
</tr>
<tr>
<td>Seawater</td>
<td>Seawater</td>
<td>37.7-40.4</td>
<td>(Foster et al., 2010)</td>
</tr>
</tbody>
</table>
Figures

Fig. 1. (a) Speciation of dissolved inorganic boron, B(OH)₃ and B(OH)₄⁻, as a function of seawater pH. (b) δ¹¹B of dissolved inorganic boron species as a function of seawater pH. The pKₐ is 8.6 at 25 °C and 35 psu (Dickon, 1990), α is 1.0272 (Klochko et al., 2006), and δ¹¹B_SW is 39.61 (Foster et al., 2010).

Fig. 2. Examples of previously published δ¹¹B_{CaCO₃}-pH_SW trends for corals, foraminifera and brachiopods. Although many B isotope data sets are available, only studies with ≥3 δ¹¹B_{CaCO₃}-pH_SW data spanning a sufficiently wide range of pH_SW conditions were selected to illustrate the range of δ¹¹B_{CaCO₃}-pH_SW trends published to date. The two grey lines correspond to the theoretical seawater borate δ¹¹B-pH curves that have been applied most frequently to interpret δ¹¹B variability in marine calcifiers. The pKₐ 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) used in the ion exchange column.

Fig. 4. Boron isotopic composition (± SD) of different species of marine calcifiers as a function of seawater pH (± SD). The six species shown in this figure were grown under controlled pCO₂ conditions of ca. 409 ppm. Grey lines are theoretical seawater δ¹¹B_{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 pKₐ of 8.6152 at 25°C and 32 psu). Although α = 1.0272 (Klochko et al., 2006) is presently the most commonly used α, δ¹¹B_{B(OH)₄⁻}-pH curves calculated from other values of α are also shown for comparison.

Fig. 5. Exploring the potential influence of pH and boron speciation on δ¹¹B_{CaCO₃} (adapted from Rollion-Bard et al., 2011b). The solid and dashed curves represent the δ¹¹B_{CaCO₃} 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 all species examined, only the coralline algae has a δ¹¹B_{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; see text for details).
Author Contribution

RAE and JBR conceived of the project and wrote the proposals that funded the work. JBR performed the culturing 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. 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.
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[B(OH)$_3$]/[B(OH)$_4^-$] (µmol/kg)

δ$^1$B (‰)

pH

Seawater

(a)

(b)
Seawater borate

- \( \alpha = 1.0194 \) (Kakahana et al., 1977)
- \( \alpha = 1.0272 \) (Klochko et al., 2006)

- Acropora nobilis (Hönisch et al., 2004)
- Porites cylindrica (Hönisch et al., 2004)
- Stylophora pistillata (Holcomb et al., 2014)
- Orbulina universa (Sanyal et al., 1996)
- Globigerinoides ruber (Henehan et al., 2013)
- Brachiopod (Penman et al., 2013)
Figure 1: Elution profile of boron for three different volumes of Amberlite resin (60 µL, 250 µL, 500 µL).
Seawater borate
$\alpha = 1.0272$ (Klochko et al., 2006)

Seawater borate
$\alpha = 1.0285$ (Byrne et al., 2006)

Seawater borate
$\alpha = 1.0330$ (Palmer et al., 1987)
The graph shows the relationship between pH and δ¹¹B (%00) for different species. The pH range is from 7.0 to 10.0, and the δ¹¹B range is from 0% to 100% B(OH)₃. The graph includes markers for:

- Temperate coral
- Serpulid
- Coralline red alga
- Tropical urchin
- Temperate urchin
- American oyster