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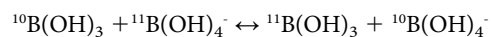
Red coralline algae assessed as marine pH proxies using ^{11}B MAS NMRSUBJECT AREAS:
BIOGEOCHEMISTRY
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Reconstructing pH from biogenic carbonates using boron isotopic compositions relies on the assumption that only borate, and no boric acid, is present. Red coralline algae are frequently used in palaeoenvironmental reconstruction due to their widespread distribution and regular banding frequency. Prior to undertaking pH reconstructions using red coralline algae we tested the boron composition of the red coralline alga *Lithothamnion glaciale* using high field NMR. In bulk analysed samples, thirty percent of boron was present as boric acid. We suggest that prior to reconstructing pH using coralline algae 1) species-specific boron compositions and 2) within-skeleton special distributions of boron are determined for multiple species. This will enable site selective boron analyses to be conducted validating coralline algae as palaeo-pH proxies based on boron isotopic compositions.

Concerns about current and projected ocean acidification as a consequence of oceanic uptake of anthropogenic atmospheric CO_2 ¹, heighten our need to understand past oceanic pH. Boron isotopic composition ($\delta^{11}\text{B}$) of marine biogenic carbonates has been considered an attractive pH proxy for several decades since the early assessments using foraminifera^{2–3}. As a marine pH proxy, $\delta^{11}\text{B}$ has already provided insight using several marine biomineralisers including much further work on foraminifera^{4–9}, corals^{10–14} and brachiopods^{15–17}.

Free-living (maerl / rhodoliths) and encrusting (crustose coralline algae) red coralline algae are attractive marine pH proxy organisms since they have a global pole to pole distribution providing an outstanding spatial data source that spans a range of water temperatures and salinities. In addition, the growth mechanism of coralline algae whereby new growth occurs on top of old provides a rich temporal record^{18–19}. The high Mg-calcite of coralline algae records ambient seawater temperature^{18,20} with the seasonal growth bands (Figure 1) providing the potential for a pH calendar.

In the ocean, boron exists in two molecular species: trigonal boric acid ($\text{B}(\text{OH})_3$) and tetrahedral borate ($\text{B}(\text{OH})_4^-$). From here on in, boric acid and borate are referred to as B3 and B4 respectively. The ratio of B3 to B4 is sensitive to pH with B3 being more abundant at low pH^{2,6,8,21}. There are two isotopes of B: ^{11}B and ^{10}B with ^{11}B being four times as abundant as ^{10}B . The isotopic fractionation between ^{11}B and ^{10}B in the dissolved species can be represented by the following mass balance:



Boric acid is enriched in ^{11}B relative to borate ion by 27.2‰²². As proportions of $\text{B}(\text{OH})_3$ and $\text{B}(\text{OH})_4^-$ vary, so will the isotopic composition such that $\delta^{11}\text{B}$ in marine carbonates is a function of pH, assuming that only $\text{B}(\text{OH})_4^-$ is incorporated into marine carbonates². For any biomineral proxy organism, this sole incorporation of B4 is the key assumption to be verified^{23–24}. In the case of coralline algae the combination of high resolution spatial and temporal data cannot be exploited until this assumption is checked. If it is the case that only B4 is incorporated into the coralline algal skeleton then the B/Ca ratio and $\delta^{11}\text{B}$ may be used to determine past ocean pH in coralline algae as in some species of planktonic foraminifera⁴ or in coral skeleton^{25–26}. ^{11}B magnetic angle spinning nuclear magnetic resonance (MAS NMR) has proved to be a powerful probe of boron speciation in biogenic carbonates^{10,27}. Compared to previous studies, the experiments reported here have been performed at very high magnetic field (18.8 T), leading to highly resolved spectra with well-separated B3 and B4 regions. Moreover, the experiments have been conducted on a prototype measurement probehead equipped with a B-free stator, leading to ^{11}B background free spectra. For the first time, the combination of high field and ^{11}B background free spectra allows the investigation of the ^{11}B speciation in biogenic carbonate with efficient and standard single pulse acquisitions. The B3/B4 quantification was then derived from standard signal integration whereas previous studies required ^{11}B background signal subtraction¹⁰ or complex signal simulation²⁷ that introduced error in the B3/B4 ratio determination. Thus, high field MAS NMR is applied here

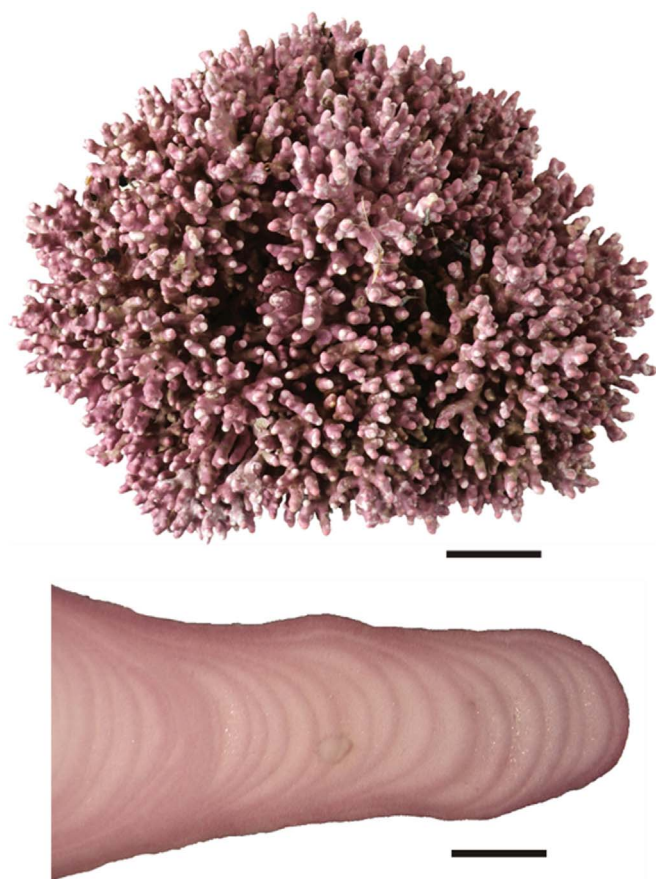


Figure 1 | Thallus and branch of maerl, *Lithothamnion glaciale*. (a) Thallus of *Lithothamnion glaciale* (scale bar = 1 cm), (b) transverse section through a *L. glaciale* branch tip indicating regular banding (scale bar = 1.5 mm).

to red coralline algae as a primary assessment of the suitability of this globally distributed biogenic carbonate as a marine pH recorder.

Results

MAS NMR performed at high field (18.8 T) readily resolves the B3 and B4 signals in *L. glaciale* (Figure 2). In addition to the main B3 (18 ppm) and B4 (2 ppm) signals, a third resonance at 6 ppm can be observed, indicating that a second B4 species is present at low amount inside the samples. In *L. glaciale* with and without epithelium present, boron in the trigonal form (B3) accounts for around 30% of the boron present and B3 is thus present in the calcified high Mg skeleton (Table 1). Boron in the tetragonal forms (B4) account for around 70% with 64% originate from the major B4 unit at 2 ppm and 6% from the minor B4 unit at 6 ppm. This finding indicates that the assumption of only tetrahedral boron (B4) being incorporated into biogenic carbonates required for pH reconstruction is not met on a whole-branch scale in coralline algae, since it is clear that a significant amount of trigonal boron (B3) is present. This incorporation of a significant proportion of B3 has occurred in *L. glaciale* from Loch Sween where the mean annual pH is 8.10. In these conditions, the proportion of B3 to B4 in seawater is around 4:1^{6,817}. Thus, the proportion of B3 in *L. glaciale* does not reflect the direct proportion of B3 relative to B4 in seawater, B4 being majority incorporated into the skeleton. Importantly, lower pH in ocean acidification conditions would increase the proportion of B3 to B4 in seawater.

Discussion

A primary assessment of the pH-reconstruction assumption that boron is incorporated into coralline algae solely in the tetrahedral (B4) form was made using powdered whole branches. Both trigonal

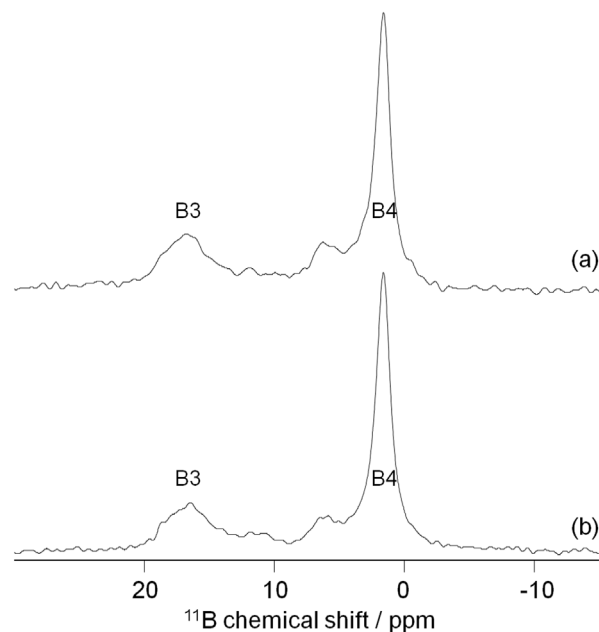


Figure 2 | Magnetic angle spinning nuclear magnetic resonance spectra of *Lithothamnion glaciale*. High field (18.8 T)¹¹B MAS-NMR analyses of two samples of powdered *L. glaciale* (a) with and (b) without epithelium, indicating trigonal (B3) and tetrahedral (B4) boron.

and tetrahedral boron were present; had high-field NMR indicated that all boron was in the tetrahedral form, then *L. glaciale* could then be used in bulk powdered form to determine past oceanic pH. Boric acid is not localised just in the epithelium, but is present in the high Mg skeleton as well. The presence of B3 was already observed in corals and in foraminifera^{9,19}. This presence could account for some of the vital effects in the boron isotopic compositions of biocarbonates, together with an elevation of pH at the sites of calcification²⁸. Indeed, some species of coralline algae can increase their internal pH up to 0.5 pH units in light conditions²⁹. It is clear that a bulk approach cannot be pursued for pH reconstruction and likely indicates that spatially non-selective analysis of within growth band CaCO₃ may also be sampling both B3 and B4. To account for the presence of B3, it is possible to adjust calculations allowing for a 30% B3 presence. However, prior to this, four further checks should be conducted:

1. Test boron composition in other coralline algal species to determine whether *L. glaciale* portrays the exception or the rule.
2. Skeletal spatially-specific testing of *L. glaciale* and other coralline algal species investigating winter and summer growth bands separately since the faster growth rate of summer growth and slower winter growth may result in different rates of B3 incorporation. Faster growth rates in summer occur in tandem with more Mg incorporation¹⁸ and this could be associated with more or less B3 incorporation. Slow winter growth may exclude B3 and thus the calcite deposited during winter may provide a more ideal pH proxy. Understanding this is critical in the light of observations in other organisms where B3 incorporation is not uniform throughout biomineral structures e.g. as in corals¹⁰.
3. Quantification of B3 incorporation in *L. glaciale* grown in different pH environments to determine any pH-dependent variation in B3 incorporation to determine whether B3 is incorporated directly into the skeleton or if B4 is modified to B3 after boron incorporation as suggested by²⁷.
4. Measurement of $\delta^{11}\text{B}$ to test if B3 is incorporated from seawater or is due to a change of B4 after the carbonate precipitation as proposed by²⁷.

Species-specific boron compositions and within-skeleton special distributions of boron are determined for multiple species of


Table 1 | Proportion of trigonal (B3) and tetrahedral (B4) boron in *Lithothamnion glaciale* with (+) and without (-) epithelium

<i>L. glaciale</i>	B3 (%)	B4 (%)
(a) + epithelium	30.3 ± 4.3	(62.7 + 7.0) ± 4.3
(b) - epithelium	27.7 ± 4.6	(66.5 + 5.8) ± 4.6

coralline algae. This will enable site selective boron analyses to be conducted validating coralline algae as palaeo-pH proxies based on boron isotopic compositions.

Methods

Duplicate specimens of a single species of free-living red coralline algae, *Lithothamnion glaciale* were collected from Loch Sween, Scotland 56°01.99'N, 05°36.13'W from 5 m depth using SCUBA. Annual temperature range at the collection site is 6–17°C and average pH is 8.10 (measured *in situ* with YSI EXO2 Sonde equipped with pH probe which was calibrated monthly and drift adjusted).

Branches of two air-dried specimens were brushed to remove any ground in material and powdered using a Retsh MM400 ball mill. One thallus was crushed with the outer epithelium in place while the second thallus had the epithelium removed using a sanding drill (Dremmel Tool) prior to crushing.

¹¹B magic angle spinning nuclear magnetic resonance (MAS-NMR) experiments were carried out at 256.71 MHz on a Bruker Avance III spectrometer. A 3.2 mm probehead, operating at a spinning frequency of 20 kHz and equipped with a Vespel® stator (avoiding the presence of probe background signal in the ¹¹B NMR experiments) has been used. The spectra were acquired with 0.5 μs pulse length (10° pulse angle) and 700 k transients separated by a 0.2 s recycle delay, leading to a complete measuring time of 40 hours. The trigonal (B3) to tetrahedral (B4) ratios were directly extracted from the spectra by standard signal integration and errors were determined from noise signal integration.

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Author contributions

M.C. and N.A.K. conceived the project, N.K. prepared the samples, C.R.-B. And G.T. conducted the NMR analyses, N.K. prepared Figure 1 and G.T. prepared Figure 2, M.C. wrote the main manuscript and all authors contributed to the final manuscript.

Additional information

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