Coralline algal Mg-O bond strength as a marine pCO$_2$ proxy

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Full Methods:

Sample Collection

*Lithothamnion glaciale* is a red free-living photosynthetic alga which lays down a high Mg calcite skeleton (Kamenos et al., 2008) (Fig. 1). Coralline algae (*Lithothamnion glaciale*) were collected in summer 2011 from Loch Sween, Scotland (56°01.99'N, 05°36.13'W) (Fig. 2) using SCUBA.

Sample Incubation

Algal thalli were stained with Calcein (Sigma-Aldrich), a fluorescent dye (24h at 50µM concentration (Brahmi et al., 2010)), to enable identification of skeletal material deposited during incubation (Fig 1). Following staining, thalli were grown for 24 months in the Marine Mesocosm Facility at the University of Glasgow (School of Geographical and Earth Sciences). Mesocosms followed the same ambient temperatures (4.5°C during winter to 17.5°C during summer), day length and photosynthetically active radiation (90µmol photons m$^{-2}$ s$^{-1}$) as that at the collection site (Rix et al., 2012). During incubation, thalli were exposed to one of three pCO$_2$ treatments (per treatment: n = 5 thalli, n = 2 mesocosms):

- Control treatment: 380µatm pCO$_2$
- Moderate acidification: 750µatm pCO$_2$
- High acidification: 1000µatm pCO$_2$

Experimental mesocosms were acidified by CO$_2$ bubbling at the desired concentrations. CO$_2$ concentrations were monitored using LI-COR 820 gas analysers.

Carbonate chemistry parameters

During incubation, daily seawater temperature, salinity, dissolved oxygen (YSI Pro2020) and pH (Mettler-Toledo MA235) were monitored. Every two weeks, water samples were preserved for
carbonate chemistry with MgCl\(_2\) (Dickson et al., 2007) and stored in the dark for subsequent total alkalinity (TA) and dissolved inorganic carbon (DIC) analysis. DIC was determined using a CO\(_2\) Coulometer (CM5014 v.3, UIC Ltd at the Scottish Association for Marine Science) and methods described by (Dickson et al., 2007). TA was determined using the 2-stage open-cell titration method (Dickson et al., 2007) at the University of Glasgow. In situ TA, DIC, temperature and salinity measurements were used to calculate pH, pCO\(_2\), HCO\(_3^-\) and CO\(_3^{2-}\) concentrations and calcite (\(\Omega_{\text{Calcite}}\)) and aragonite saturation states using CO2SYS (Pierrot et al., 2006) with dissociation constants from (Mehrbach et al., 1973) refit by Dickson and Millero (Dickson and Millero, 1987) and [KSO\(_4\)] using (Dickson, 1990) (Table DR1).

**Sample Preparation**

Following 24 months of growth within treatment mesocosms, branches from different *L. glaciale* individuals (n = 5) from each treatment were transverse sectioned and polished in resin blocks (Buehler Epoxycure). Growth before, and after treatment, was identified by determining the position of the Calcein stain using fluorescence microscopy (Olympus BH-2 microscope). Even though samples were grown in treatments for 24 months, to ensure we did not analyze the most recently deposited partially calcified cells, only the 12 months of growth directly after staining were considered.

**Sample Analysis**

Raman spectroscopy (Renishaw inVia Raman) was conducted following (Hennige et al., 2014; Kamenos et al., 2013) at the University of Glasgow to determine Mg concentrations and molecular bonding strength between Mg and O molecules within the high Mg calcium carbonate skeleton of *L. glaciale*. A 785 nm laser with 1200 l mm\(^{-1}\) grating and 15 µm spot size were used in a linear transect across each growth band with 2 spots being analysed per season for each replicate (n = 5). The Raman shift peak at \(~1089\text{cm}^{-1}\) corresponds to Mg concentrations within the sample structure while the full width at half peak maximum (FWHM) is linearly correlated to positional disorder of the Mg ions.
within the calcite lattice and in turn bond strength between Mg and O molecules (Bischoff et al., 1985). In *L. glaciale*, Mg is a component of the crystal lattice rather than a component of associated organics (Kamenos et al., 2009) and thus increases in Mg-O bond strength can be attributed to positional disorder of crystal lattice bringing Mg and O closer together via Mg ions moving out of the plane parallel to the a-axis in the direction of the c-axis (Bischoff et al., 1985). Sample Raman parameters were normalized (Normal-Inverse-Wishart distribution) to remove any differences in magnitude of starting parameters due to individual variability (some individuals will naturally have stronger / weaker bond strengths than others) thus enabling direct comparison between and within treatments.

**Seasonal variability:** Within microscopy micrographs, each annual growth band was digitally subdivided into 6 proportional segments to give 2-month growth intervals, across summer through seasonal transition into winter. Transition from winter to spring was not considered as in *L. glaciale* this can be quite abrupt, sometimes with little skeletal calcite deposition. This procedure was conducted on micrographs of the annual growth band deposited prior to sample collection (before the Calcein stain) and the growth band subsequent to Calcein staining which represented growth within the mesocosm treatments. The growth band prior to sample collection was also used a double control to determine any influence of sample handling on each individual.

**Between treatment variability:** Mg-O bond strength and relative Mg concentrations were compared between treatments for segments of the algal growth bands that were deposited during the same season.

**Statistical Analysis**

Relationships between bond strength and $pCO_2$ concentrations were determined using linear regression analysis. Analysis of covariance (ANCOVA) was performed to determine the influence of
$p$CO$_2$ on Mg-O bond strength with relative Mg concentration as a covariate. $p$CO$_2$ was used as the independent variable in comparisons as treatment carbonate chemistry was controlled using CO$_2$ bubbling. Regression analysis on normalized FWHM was used to determine the linear relationships between $p$CO$_2$ and Mg-O bond strength over the whole year of growth. Relative Mg concentrations (1089 cm$^{-1}$ Raman peak frequency) were included as a covariate during regression to account for temperature-driven, stage-dependent changes in lattice Mg concentrations. Standard error on calculated $p$CO$_2$ from FWHM was determined by removing (linear regression) the influence of Mg concentration (indicative of temperature) and propagating the FWHM range for each $p$CO$_2$ treatment through the remaining relationship to the $p$CO$_2$ calculation (Table 1, Fig. DR2). Data met all parametric test assumptions and regression analysis. All analyses were conducted in R v 3.03.

**Figure DR1:** Calcite crystal showing Ca$^{2+}$ replacement by Mg$^{2+}$. a) Low Mg calcite, b) 13 mol% MgCO$_3$, c) 19 mol% MgCO$_3$, d) 25 mol% MgCO$_3$. a, b and c axes indicated. Crystal generated in CrystalMaker v 9.1.0.
Figure DR2: Mean normalized FWHM for *Lithothamnion glaciale* grown in 380, 750 and 1000 µatm $pCO_2$ after the removal of temperature-induced changes in Mg concentrations. Error = SE.

Table DR1: Carbonate chemistry parameters for the 24-month incubation. Mean ± SD values for measured (n = 42) temperature, salinity, pH and alkalinity, and calculated dissolved inorganic carbon (DIC), bicarbonate (HCO$_3^-$), carbonate (CO$_3^{2-}$), pCO$_2$, calcite saturation state ($\Omega_{\text{cal}}$) and aragonite saturation state ($\Omega_{\text{arag}}$) for all sampling periods.

<table>
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<th>Temperature (°C)</th>
<th>Salinity</th>
<th>pH (T)</th>
<th>Alkalinity (µmol kg$^{-1}$)</th>
<th>DIC (µmol kg$^{-1}$)</th>
<th>HCO$_3^-$ (µmol kg$^{-1}$)</th>
<th>CO$_3^{2-}$ (µmol kg$^{-1}$)</th>
<th>pCO$_2$</th>
<th>$\Omega_{\text{cal}}$</th>
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<td>1195.1</td>
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Repository Item References


