

1 **Boron isotope sensitivity to seawater pH change in a species of *Neogoniolithon* coralline**
2 **red alga**

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11 The increase in atmospheric carbon dioxide (CO₂) observed since the industrial revolution has
12 reduced surface ocean pH by ~0.1 pH units, with further change in the oceanic system predicted
13 in the coming decades. Calcareous organisms can be negatively affected by extreme changes in
14 seawater pH (pH_{sw}) such as this due to the associated changes in the oceanic carbonate system.
15 The boron isotopic composition ($\delta^{11}\text{B}$) of biogenic carbonates has been previously used to
16 monitor pH at the calcification site (pH_{cf}) in scleractinian corals, providing mechanistic insights
17 into coral biomineralisation and the impact of variable pH_{sw} on this process. Motivated by these
18 investigations, this study examines the $\delta^{11}\text{B}$ of the high-Mg calcite skeleton of the coralline red
19 alga *Neogoniolithon* sp. to constrain pH_{cf}, and investigates how this taxon's pH_{cf} is impacted by
20 ocean acidification. $\delta^{11}\text{B}$ was measured in multiple algal replicates (n = 4 to 5) cultured at four
21 different pCO₂ scenarios – averaging ($\pm 1\sigma$) 409 (± 6), 606 (± 7), 903 (± 12) and 2856 (± 54)
22 μatm , corresponding to average pH_{sw} ($\pm 1\sigma$) of 8.19 (± 0.03), 8.05 (± 0.06), 7.91 (± 0.03) and
23 7.49 (± 0.02) respectively. Results show that skeletal $\delta^{11}\text{B}$ is elevated relative to the $\delta^{11}\text{B}$ of
24 seawater borate at all pH_{sw} treatments by up to 18 ‰. Although substantial variability in $\delta^{11}\text{B}$
25 exists between replicate samples cultured at a given pH_{sw} (smallest range = 2.32 ‰ at pH_{sw}
26 8.19, largest range = 6.08 ‰ at pH_{sw} 7.91), strong correlations are identified between $\delta^{11}\text{B}$ and
27 pH_{sw} ($R^2 = 0.72$, $p < 0.0001$, $n = 16$) and between $\delta^{11}\text{B}$ and B/Ca ($R^2 = 0.72$, $p < 0.0001$, $n = 16$).
28 Assuming that skeletal $\delta^{11}\text{B}$ reflects pH_{cf} as previously observed for scleractinian corals, the
29 average pH_{cf} across all experiments was 1.20 pH units (0.79 to 1.56) higher than pH_{sw}, with the

30 magnitude of this offset varying parabolically with decreasing pH_{sw} , with a maximum difference
31 between pH_{sw} and pH_{cf} at a pH_{sw} of 7.91. Observed relationships between pH_{sw} and calcification
32 rate, and between pH_{sw} and pH_{cf} , suggest that coralline algae exhibit some resilience to
33 moderate ocean acidification *via* increase of pH_{cf} relative to pH_{sw} in a similar manner to
34 scleractinian corals. However, these results also indicate that pH_{cf} cannot be sufficiently
35 increased by algae exposed to a larger reduction in pH_{sw} , adversely impacting calcification rates
36 of coralline red algae.

37

38 *1.0 Introduction*

39 Atmospheric CO_2 has been increasing since the Industrial Revolution, from 280 ppm to more
40 than 400 ppm today (Tans and Keeling, 2016). This increase has led to changes in ocean carbon
41 chemistry, ultimately lowering seawater pH (pH_{sw}) by 0.1 pH units. Climate models predict that
42 by 2100, a high-end “business as usual” emission scenario (*i.e.* Intergovernmental Panel on
43 Climate Change: Representative Concentration Pathway 8.5) will result in a global average
44 surface pH_{sw} of ca. 7.8, potentially reaching even lower levels at high latitudes. This large and
45 rapid reduction in global pH_{sw} will result in an environment that is potentially challenging to
46 marine organisms that rely on biogenically produced CaCO_3 (Doney et al., 2009).

47

48 Ocean acidification affects biogenic calcification by reducing the CaCO_3 saturation state of
49 seawater ($\Omega = [\text{Ca}^{2+}][\text{CO}_3^{3-}]/K_{\text{sp}}^*$; where K_{sp}^* is the stoichiometric solubility product of CaCO_3 at
50 in situ conditions of temperature, salinity and pressure). Reductions in Ω of seawater have been
51 shown to reduce calcification rates and, in some cases, cause net dissolution of the calcareous
52 shells and skeletons of marine organisms (Gattuso et al., 1998; Riebesell et al., 2000; De’ath et
53 al., 2009; Ries et al., 2009; Ries et al., 2016). Indeed, a recent study investigating a sub-marine
54 volcanic CO_2 seep as an analogue for the effects of ocean acidification found that, over time, the
55 nearby coral reef system was largely replaced by fleshy algae-covered rocks (Enochs et al.,
56 2015). This, and a wealth of other studies (Gattuso et al., 2015; and references therein), indicate

57 that ocean acidification can directly affect calcareous organisms through changing ocean
58 carbonate chemistry, as well as indirectly *via* inter-species competition and modification of
59 species interactions (*e.g.*, Dodd et al., 2015).

60

61 Coralline algae are important CaCO₃ producers and are often found in high latitude waters. They
62 also comprise a large component of modern coral reefs, confer stability to the reef crest, and are
63 a vital food source for marine grazers such as sea urchins (McCoy and Kamenos, 2015). Hence,
64 coralline algae play an important role in the marine food web, but also act as ecosystem
65 engineers by providing defence against coastal erosion. Coralline algae are predominantly
66 composed of high-Mg calcite (> 15 mol % MgCO₃), which is more soluble than aragonite or low-
67 Mg calcite found in other calcareous organisms such as corals, scallops and oysters (Ries et al.,
68 2016). Since CO₂ is more soluble in colder water, it is likely that global high latitude regions are
69 more vulnerable to ocean acidification than lower latitude regions (Gattuso et al., 2015). Thus,
70 ocean acidification poses a severe threat to coralline algae and their interdependent ecosystems
71 (Kuffner et al., 2008; Gao and Zheng, 2009; Ragazzola et al., 2012).

72

73 Coralline red algae calcify by depositing calcite within their cell walls, but exterior to their cell
74 membrane. This is in contrast to foraminifera that calcify within seawater vacuoles (Erez,
75 2003), scleractinian corals that calcify in a fluid between their skeleton and calicoblastic
76 epithelium (Cohen and McConnaughey, 2003; Gagnon et al., 2012), and coccolithophores that
77 calcify in an intracellular vesicle (Mackinder et al., 2010), but is similar to calcification within
78 *Bryopsidalean* calcareous green algae, which occurs extracellularly within interutricular space
79 (Ries, 2009).

80

81 Several studies have examined the influence of ocean acidification on the nature and rate of
82 calcification in a variety of coralline red algae (Hall-Spencer et al., 2008; Martin and Gattuso,
83 2009; Roleda et al., 2015; Cornwall et al., 2017). For instance, Ries et al. (2009) and Smith and

84 Roth (1979) documented a parabolic response in calcification rate of the coralline red algae to
85 decreasing pH_{sw} , suggesting that the algal calcification increases in response to moderate
86 elevations in pCO_2 , but decreases in response to extreme increases. However, Ries et al. (2009)
87 observed maximum calcification between pH_{sw} 7.9 and 8.1 ($\Omega_{\text{A}} \sim 2.0$ to 2.3; where Ω_{A} is the
88 saturation state for the aragonite CaCO_3 polymorph), while Smith and Roth (1979) observed
89 maximum calcification between pH_{sw} 7.6 and 8.3 (Figure 1). These non-linear relationships
90 suggest that coralline algae utilise biological processes to confer resilience to moderate-to-
91 extreme changes in pH_{sw} .

92

93 The calcification response of coralline algae to ocean acidification has been shown to vary
94 between species (Borowitzka, 1981; Semesi et al., 2009; Comeau et al., 2013). Despite this, a
95 result common to the various species investigated in the different experiments is their ability to
96 continue calcifying, albeit at slower rates, even under extremely reduced pH_{sw} . This mitigation
97 of extreme ocean acidification has been shown to translate into coralline algae survival in low
98 pH_{sw} environments across a range of natural ecosystems (Kamenos et al., 2016). Coralline red
99 algae perform both calcification and photosynthesis (*e.g.*, Buitenhuis et al., 1999), and the
100 balance between these two key biological processes is important for coralline algae survival.
101 Many marine organisms utilise carbon concentrating mechanisms intracellularly to ensure
102 calcification can still occur under CO_2 -limited conditions. Experimental work has shown that
103 photosynthesis in some marine algae is CO_2 -limited up to *ca.* 1000 μatm pCO_2 (Bowes, 1993).
104 Therefore, the additional energy from photosynthesis as pCO_2 becomes elevated up to *ca.* 1000
105 μatm may stimulate calcification within calcifying marine algae, despite the associated decrease
106 in pH_{sw} . This effect has previously been observed for zooxanthellate scleractinian corals (*e.g.*,
107 Castillo et al., 2014). Furthermore, photosynthesis increases local pH through the removal of
108 dissolved CO_2 from seawater proximal to the algae (Gao et al., 1993), and respiration may
109 reduce calcification rates by decreasing local pH as a consequence of CO_2 release (De Beer and
110 Larkum, 2001). Calcification in coralline algae is therefore likely regulated by a number of

111 important metabolic activities that influence the carbonate system within and around the algal
112 cell (Smith and Roth, 1979; Gao et al., 1993; Hurd et al., 2011; Martin et al., 2013).

113

114 The impact of pH_{sw} and CaCO_3 saturation state on inorganic calcification differs from their
115 impact on biogenic calcification (Ries et al., 2009; McCulloch et al., 2012a). The IpHRAC model
116 by McCulloch et al. (2012b) ascribes the reduced sensitivity of scleractinian coral calcification in
117 response to changing seawater aragonite saturation state to the increase of the calcification site
118 pH (pH_{cf}), as determined from the boron isotopic composition of the coral skeleton, proton-
119 sensitive microelectrodes (Ries, 2011a), and pH-sensitive dyes (Venn et al., 2013). Recent
120 studies investigating $\delta^{11}\text{B}$ of the coralline algae *Clathromorphum nereostratum* via laser ablation
121 inductively coupled plasma mass spectrometry (LA-ICPMS) reveal that skeletal $\delta^{11}\text{B}$ within this
122 species is also consistent with a pH_{cf} that is significantly higher than measured ambient pH_{sw} (by
123 ca. 0.6 pH units; $\Delta\text{pH} = \text{pH}_{\text{cf}} - \text{pH}_{\text{sw}}$), suggesting an increase of pH_{cf} may play a similarly
124 important role in coralline algal calcification (Fietzke et al., 2015). However, skeletal $\delta^{11}\text{B}$ data
125 for coralline algae species cultured under a range of controlled pH_{sw} conditions that
126 demonstrate the response of pH_{cf} to changes in pH_{sw} are currently sparse (*e.g.* the only other
127 such study is Cornwall et al., 2017). Here, the boron isotope approach to estimating pH_{cf} is
128 applied to a branched *Neogoniolithon* sp. cultured under four pCO_2 conditions that allow us to
129 assess the potential impacts of ocean acidification on pH_{cf} regulation in coralline red algae.

130

131 2.0 Methods

132 2.1 Boron isotopes

133 Numerous papers have presented detailed discussions about the basis for the boron isotope
134 proxy of pH_{sw} (Hemming and Hanson, 1992; Zeebe and Wolf-Gladrow, 2001; Foster and Rae,
135 2016). Briefly, the proxy arises because (1) the abundance of the two major aqueous forms of
136 boron in seawater are pH dependent and (2) there is boron isotope fractionation between these
137 two boron species (Dickson, 1990). Trigonal planar boric acid ($\text{B}(\text{OH})_3$) dominates at low pH,

138 and the tetrahedral tetrahydroxyborate anion ($B(OH)_4^-$; henceforth referred to as borate)
 139 dominates when pH exceeds 8.6 in typical surface ocean conditions. The two stable isotopes of
 140 boron (^{10}B and ^{11}B) occur roughly in a 1:4 ratio, and the structural difference between the
 141 aqueous species leads to an enrichment of ^{11}B in boric acid of approximately 27.2 ‰ (Klochko
 142 et al., 2006; Nir et al., 2015) because the more stable trigonal structure has the stronger B-O
 143 bonds. Boron isotopic composition is described using the delta notation $\delta^{11}B$ relative to a boric
 144 acid standard (NIST SRM 951 boric acid according to Catanzaro et al., 1970) shown in equation
 145 (1).

$$146 \quad \delta^{11}B \text{ (‰)} = \left[\left(\frac{{}^{11}B/{}^{10}B_{sample}}{{}^{11}B/{}^{10}B_{standard}} \right) - 1 \right] \times 1000 \quad (1)$$

147 Since the $\delta^{11}B$ of total boron in seawater (boric acid and borate) is constant at 39.61 ± 0.04 ‰
 148 (Foster et al., 2010), as the proportions of boric acid and borate change with pH_{sw} , the $\delta^{11}B$
 149 composition of each species also varies as a function of pH, with borate $\delta^{11}B$ increasing with
 150 pH_{sw} as described in equation (2).

$$151 \quad \delta^{11}B_{B(OH)_4^-} = \frac{\delta^{11}B_{sw} + (\delta^{11}B_{sw} - 1000(\alpha_B - 1)) 10^{pK_B^* - pH}}{1 + \alpha_B 10^{pK_B^* - pH}} \quad (2)$$

152 Where pK_B^* is the dissociation constant (dependent on temperature and salinity; Dickson,
 153 1990), $\delta^{11}B_{sw}$ is the $\delta^{11}B$ composition of total boron in seawater, $\delta^{11}B_{B(OH)_4^-}$ is the $\delta^{11}B$
 154 composition of aqueous borate, and α_B is a constant (1.0272; Klochko et al., 2006) describing
 155 the equilibrium mass dependent boron isotope fractionation between boric acid and borate.

156

157 Although borate is assumed to be the most likely form of aqueous boron incorporated into
 158 $CaCO_3$, the $\delta^{11}B$ of many biogenic carbonates is elevated relative to the $\delta^{11}B$ of seawater borate
 159 (Figure 2 and references therein; see also Vengosh et al., 1991; Gaillardet and Allègre, 1995). As
 160 noted above, this increase in the $\delta^{11}B$ of scleractinian deep-sea and tropical corals is thought to
 161 be predominantly caused by the elevation of pH_{cf} *via* enzymatic activity (*e.g.* Ca-ATPase;
 162 McConnaughey and Falk, 1991). In this case, pH_{cf} can be calculated using boron isotopes by
 163 substituting $\delta^{11}B$ of the coral sample for $\delta^{11}B$ of aqueous borate in equation (3).

$$pH = pK_B^* - \log \left(- \frac{\delta^{11}B_{sw} - \delta^{11}B_{B(OH)_4^-}}{\delta^{11}B_{sw} - \alpha_B \delta^{11}B_{B(OH)_4^-} 1000(\alpha_B - 1)} \right) \quad (3)$$

Figure 2 shows $\delta^{11}B$ data from previous studies for several coral taxa grown over a range of pH_{sw} conditions (Hönisch et al., 2004; Reynaud et al., 2004; Krief et al., 2010; Anagnostou et al., 2012; McCulloch et al., 2012b; Holcomb et al., 2014). In all cases pH_{cf} is elevated by around 0.5 pH units at pH_{sw} 8, which is similar to observations of the calcifying fluid from micro-electrodes (Al-Horani et al., 2003; Krief et al., 2010; Ries, 2011a; Trotter et al., 2011; McCulloch et al., 2012b) and pH sensitive dyes (Venn et al., 2011; Venn et al., 2013; Holcomb et al., 2014). Furthermore, the majority of corals examined thus far show that as ambient pH_{sw} decreases, pH_{cf} declines at a reduced rate (Venn et al., 2011; Venn et al., 2013; Holcomb et al., 2014).

2.2 Algal Culture

A single species of tropical coralline red alga, *Neogoniolithon* sp., was cultured at four pCO_2 ($\pm 1\sigma$) levels: 409 (± 6), 606 (± 7), 903 (± 12) and 2856 (± 54) μatm , resulting in pH_{sw} values ($\pm 1\sigma$) of 8.19 (± 0.03), 8.05 (± 0.06), 7.91 (± 0.03) and 7.49 (± 0.02), respectively (Ries et al., 2009). The algae were grown for 60 days in 38 L aquaria in filtered Atlantic Ocean seawater (0.2 μm ; Cape Cod, Massachusetts). The cultures were maintained at average aragonite saturation states ($\pm 1\sigma$) of 3.12 (± 0.22), 2.40 (± 0.42), 1.84 (± 0.13) and 0.90 (± 0.05), and temperatures of 25°C using 50 W electric heaters, and illuminated on a 10hr:14hr light:dark cycle. This species of coralline red algae exhibited an apparent parabolic calcification response to increasing pCO_2 , with net calcification rate increasing with an increase in pCO_2 from 409 to 606 μatm , and declining with an increase in pCO_2 to 903 and 2856 μatm (see Ries et al., 2009 and Table SM1 in the supplementary materials for further details; Figure 1).

2.3 Sample Preparation

Neogoniolithon sp. is a non-geniculate branched rhodolith form of coralline red algae. Replicate specimens were analysed for boron isotope composition at each culture pH_{sw} ($n = 5$ for pH_{sw}

190 7.91, and $n = 4$ for pH_{sw} 8.19, 8.05 and 7.49). Duplicate analyses were performed on all replicate
191 specimens except those from the pH_{sw} 7.49 treatment, due to the small mass of CaCO_3
192 mineralised under these high- $p\text{CO}_2$ conditions. Skeletal material produced exclusively under the
193 experimental treatments was identified relative to a ^{137}Ba isotope marker emplaced in the
194 skeletons at the start of the experiment (Ries, 2011b). Branches of the specimens were
195 powdered using a pestle and mortar in a clean laboratory fitted with boron-free HEPA filters at
196 the University of Southampton to produce homogenous bulk sample replicates for each
197 specimen. Following previous studies (Foster, 2008; Krief et al., 2010), approximately 3 mg of
198 each sample was cleaned using 500 μl of an oxidative mixture of 10% hydrogen peroxide (H_2O_2)
199 buffered with 0.1 M ammonium hydroxide (NH_4OH). The samples were heated in a water bath
200 and briefly ultra-sonicated a total of six times. The oxidative mixture was removed, and the
201 samples were rinsed and transferred to clean plastic vials. The samples were leached in 0.0005
202 M nitric acid (HNO_3) and then dissolved in a minimal volume of 0.5 M HNO_3 .

203

204 *2.4 Trace element and isotopic analysis*

205 Oxidatively cleaned and dissolved samples were transferred to Teflon vials and a 7% aliquot
206 was removed for trace element analysis. Elemental analysis (B/Ca and Sr/Ca) of matrix-
207 matched sample solutions was performed using ICPMS on a *Thermo Scientific Element 2* mass
208 spectrometer following the protocol of Henehan et al. (2015). Replicates of well-characterised
209 solution consistency standards measured during this study are precise to $\pm 5.6\%$ and $\pm 2.0\%$ for
210 B/Ca and Sr/Ca (95% confidence), respectively.

211

212 The remainder of each dissolved sample was reserved for boron isotope analysis and processed
213 at the University of Southampton according to well-established methods (Foster, 2008).

214 Samples were passed through micro-columns containing the boron-specific anion exchange
215 resin Amberlite IRA-743 and boron was eluted in Teflon distilled 0.5M HNO_3 . Boron isotopic
216 composition of each purified sample was then measured using a *Thermo Scientific Neptune*

217 multi-collector ICPMS (MC-ICPMS) using two Faraday detectors fitted with $10^{12} \Omega$ resistors at
218 the University of Southampton following methods detailed in Henehan et al. (2013) and Foster
219 et al. (2013). Samples were bracketed with NIST SRM 951 standard boric acid to correct for
220 variability in instrument induced mass fractionation. The long-term reproducibility of standards
221 is approximately $\pm 0.2 \text{ ‰}$ for 20 ng of boron (95% confidence), and analytical uncertainty is
222 described by equation (4), where $[^{11}\text{B}]$ is the voltage measured on the H3 faraday detector with
223 one of the $10^{12} \Omega$ resistors.

$$224 \quad 2\sigma = 12960e^{(-212[^{11}\text{B}])} + 0.3385 e^{(-1.544[^{11}\text{B}])} \quad (4)$$

225

226 3.0 Results

227 The coralline algae across all pH treatments yield $\delta^{11}\text{B}$ values ranging from $24.42 (\pm 0.22) \text{ ‰}$ to
228 $36.26 (\pm 0.10) \text{ ‰}$ (Table 1). One sample replicate at $\text{pH}_{\text{sw}} 8.19$ was deemed anomalous, as
229 duplicate analyses differed by 1.4 ‰ compared with an average difference between other
230 duplicate analyses of 0.18 ‰ . This outlying sample is therefore excluded from the discussion,
231 and $n = 16$ for all subsequent regression analyses.

232

233 The range of $\delta^{11}\text{B}$ for each pH treatment varies from 2.3 ‰ at $\text{pH}_{\text{sw}} 8.19$ to 6.1 ‰ at $\text{pH}_{\text{sw}} 7.91$.
234 The relationship between $\delta^{11}\text{B}$ of *Neogoniolithon* sp. calcite and pH_{sw} (Figure 2) demonstrates
235 that all $\delta^{11}\text{B}$ measurements in this study lie considerably above the pH_{sw} vs. aqueous borate $\delta^{11}\text{B}$
236 curve (Klochko et al., 2006), and are also elevated compared to other examples of biogenic
237 carbonates thus far quantified (McCulloch et al., 2012b), with the exception of some deep-sea
238 scleractinian corals (e.g. Blamart et al., 2007). The high $\delta^{11}\text{B}$ compositions observed in this study
239 of a branching species of *Neogoniolithon* are also similar to those found in a crustose species of
240 the same genus (Cornwall et al., 2017), suggesting that closely related species of coralline algae
241 exhibit similar boron isotope systematics and pH_{cf} , and that growth form (i.e. crustose vs.
242 branching) alone does not necessarily impart large differences in these systems. Although the

243 offset of the algae's pH_{sw} vs. $\delta^{11}\text{B}$ curve from the pH_{sw} vs. aqueous borate $\delta^{11}\text{B}$ curve is generally
244 consistent with the offset previously observed for corals grown at various pH_{sw} (Hönisch et al.,
245 2004; Reynaud et al., 2004; Krief et al., 2010; Anagnostou et al., 2012; McCulloch et al., 2012a;
246 Holcomb et al., 2014), the pH_{sw} vs. $\delta^{11}\text{B}$ relationship for the algae is better fit (with respect to
247 minimising residuals) with a parabolic model ($R^2 = 0.73$ and $p < 0.001$, vs. $R^2 = 0.53$ and $p < 0.01$
248 for linear fit) while the pH_{sw} vs. $\delta^{11}\text{B}$ relationships for corals are better fit with linear models
249 (Trotter et al., 2011; McCulloch et al., 2012b; Holcomb et al., 2014). Details of all regressions,
250 gradients and intercepts can be found in Table SM2 in the supplementary materials.

251

252 The measured B/Ca and $\delta^{11}\text{B}$ compositions are also highly linearly correlated ($R^2 = 0.77$, $p <$
253 0.0001 ; Figure 3A), a trend that is predicted from boron isotope systematics yet rarely observed
254 so clearly in biogenic carbonates (Foster, 2008; Henahan et al., 2015) with the possible
255 exception of recent work with deep-sea corals (Stewart et al., 2016). Sr/Ca has significant
256 negative correlation with $\delta^{11}\text{B}$ ($R^2 = 0.33$, $p < 0.05$; Figure 3B).

257

258 Following the interpretations of $\delta^{11}\text{B}$ in corals (Hemming et al., 1998; Rollion-Bard et al., 2003;
259 Allison and Finch, 2010; Rollion-Bard et al., 2011; McCulloch et al., 2012b), pH_{cf} calculated using
260 equation 3 (assuming boron in the algal calcite is sourced solely from seawater borate) reveals
261 an elevation of pH_{cf} relative to pH_{sw} by an average of 1.20 (± 0.22) pH units (Figure 4A). There is
262 a statistically significant linear positive correlation ($R^2 = 0.45$, $p < 0.01$) between pH_{cf} and pH_{sw} ,
263 although once again a second-order polynomial model with an optimum near pH_{sw} 7.95 better
264 describes the data ($R^2 = 0.66$, $p < 0.001$). If this model of boron incorporation is correct, ΔpH
265 plotted against pH_{sw} exhibits an apparent parabolic relationship with pH_{sw} ($R^2 = 0.46$, $p < 0.01$;
266 Figure 4B). ΔpH approaches a maximum mean of 1.26 pH units under the second most acidic
267 treatment, and although these measurements fall within 1σ of each mean, there is a significant
268 reduction of ΔpH at the most acidic treatment (pH_{sw} 7.49). For instance, t-tests reveal there is a

269 significant difference between the mean $\delta^{11}\text{B}$ composition of the algae cultured at pH_{sw} 8.19,
270 8.05 and 7.91 when compared to the algae cultured at pH_{sw} 7.49, confirming that a reduction in
271 pH_{sw} causes a decrease in pH_{cf} of coralline red algae.

272

273 The B/Ca of the algal specimens range from 352 (± 18) to 670 (± 84) $\mu\text{mol mol}^{-1}$ (Figure 5B),
274 and is therefore comparable to B/Ca in scleractinian corals, but exceeds that found in
275 coccolithophores (Stoll et al., 2012) and foraminifera (Henehan et al., 2015). Although both
276 linear ($R^2 = 0.49$, $p < 0.01$) and second-order polynomial regressions ($R^2 = 0.72$, $p < 0.001$) of the
277 B/Ca vs. pH_{sw} data are statistically significant, the polynomial model better describes the data
278 (lower p-value and higher R^2). Ranges within treatments vary from 182 $\mu\text{mol mol}^{-1}$ at pH_{sw} 7.91
279 to 52 $\mu\text{mol mol}^{-1}$ at pH_{sw} 7.49.

280

281 Calcite Sr/Ca ranges from 2.85 (± 0.10) to 3.54 (± 0.13) mmol mol^{-1} and exhibits a statistically
282 significant negative linear correlation with pH_{sw} ($R^2 = 0.59$, $p < 0.001$; Figure 5A). A negative
283 trend is also observed between Sr/Ca and B/Ca, although it is just outside of significance at the
284 95% level ($R^2 = 0.22$, $p = 0.06$; Figure 5C).

285

286 *4.0 Discussion*

287 *4.1 $\delta^{11}\text{B}$ and B/Ca as tracers of pH*

288 The boron isotope palaeo-pH proxy has been primarily applied to foraminifera, and tropical and
289 deep-sea corals (*e.g.* Spivack et al., 1993; Sanyal et al., 1996; Palmer, 1998; Krief et al., 2010; Rae
290 et al., 2011; Anagnostou et al., 2012; Henehan et al., 2013). Calcification in foraminifera occurs
291 *via* vacuolisation of seawater (Erez, 2003; de Nooijer et al., 2014), while corals are thought to
292 biomineralise from a discrete fluid between their calicoblastic epithelium and skeleton (Cohen
293 and McConnaughey, 2003). As outlined above, calcification in coralline algae occurs
294 extracellularly within and between the cell walls of the algae yet external to their cell membrane
295 (Ries, 2009). The application of the foraminifera or coral model for the $\delta^{11}\text{B}$ proxy in coralline

296 algae therefore requires some key assumptions, including in particular that the algal
297 calcification fluid has a total $\delta^{11}\text{B}$ and salinity similar to that of ambient seawater. Nonetheless,
298 recent studies have shown that calcein, which cannot be transported across cellular
299 membranes, is incorporated into the skeleton of the coralline algae *Lithothamnion glaciale*
300 (Pauly et al., 2015), supporting the assumption that the site of calcification in coralline algae is
301 at least partially open to seawater exchange (Comeau et al., 2012; Adey et al., 2013).

302

303 Regardless of the precise mechanism of calcification within this species, the strong positive
304 correlations observed here between $\delta^{11}\text{B}$ composition, B/Ca ratio and pH_{sw} indicate that boron
305 systematics of coralline algae do vary with respect to pH_{sw} . As expected from the existing
306 understanding of the proxy, cultures at lower pH_{sw} have lower $\delta^{11}\text{B}$ and B/Ca; both, in theory,
307 resulting from a reduction in borate concentration relative to boric acid at lower pH_{sw} .
308 Nonetheless, the $\delta^{11}\text{B}$ data for the coralline algae presented here plot well above the borate $\delta^{11}\text{B}$
309 vs. pH_{sw} curve. Therefore, following the model for boron isotopes in corals proposed by
310 McCulloch et al., (2012a), the results of our study suggest that coralline algae substantially
311 increase pH_{cf} to promote calcification. Indeed pH_{cf} has been shown to increase during seasonal
312 variations in ΔpH of 0.5 to 0.7 pH units within the coralline algae species *Clathromorphum*
313 *nereostratum* (Fietzke et al., 2015), and the more recent study by Cornwall et al. (2017) shows
314 that a crustose species of the *Neogoniolithon* genus exhibits a ΔpH of ca. 0.8 – 1.1 pH units,
315 depending on pH_{sw} .

316

317 Interpreting the results of the boron isotope data presented here following standard boron
318 isotope pH proxy assumptions that (1) boron enters the algal calcification site unfractionated
319 from seawater, (2) boron isotope fractionation in coralline algae is controlled only by pH_{cf} , and
320 (3) only seawater borate is incorporated into the coralline algal skeleton, suggests that
321 *Neogoniolithon* sp. undergoes a large pH_{cf} increase of, on average, 1.20 units (Figure 4). In light
322 of these findings, and the unique calcification mechanism in coralline algae compared to other

323 marine calcifiers, some alternative models of boron systematics within coralline algae should be
324 explored to ensure that these standard assumptions are met in coralline algae. The impact of
325 possible boric acid incorporation, and Rayleigh fractionation of the calcifying medium and other
326 processes affecting coralline algal skeletal chemistry are discussed in the following sections.

327

328 4.1.1 Boric acid incorporation

329 Isotopically heavy boric acid has a similar size and the same trigonal planar structure as the
330 carbonate ion (CO_3^{2-}) found in the algal calcite lattice and, whilst boric acid holds no charge, it
331 may be incorporated as an impurity. Solid state ^{11}B nuclear magnetic resonance (NMR)
332 spectroscopy on coralline algal calcite has revealed that approximately 30% of boron is present
333 in a trigonal geometry, and Cusack et al. (2015) suggested that boric acid may therefore be
334 directly incorporated into the high-Mg calcite of *Neogoniolithon* sp. The incorporation of ^{11}B
335 enriched boric acid into the calcite lattice would result in higher skeletal $\delta^{11}\text{B}$. Therefore, boric
336 acid incorporation may partially explain the positive shift in skeletal $\delta^{11}\text{B}$ compositions (relative
337 to $\delta^{11}\text{B}$ of seawater borate) that we report here (Figure 2).

338

339 Assuming that both seawater borate and boric acid are incorporated into coralline algal calcite,
340 the proportion of boric acid required to match the mean skeletal $\delta^{11}\text{B}$ compositions of the algae
341 is between 44 and 60% (Table 2), thereby greatly exceeding the ~30% suggested from *in situ*
342 ^{11}B MAS NMR studies (Cusack et al., 2015), yet it should also be noted that Cusack et al. (2015)
343 examined a different species of coralline algae (*Lithothamnion glaciale*). Furthermore, given that
344 the abundance of boric acid is pH dependent, it would be expected that the percentage of boric
345 acid incorporated should increase with decreasing pH_{sw} (*i.e.* with increasing boric acid in
346 solution; Noireaux et al., 2015). This was not observed for the specimens of *Neogoniolithon* sp.
347 investigated here, as the percentage of boric acid incorporation required to explain the ^{11}B
348 enrichment levels off for the two lowest pH_{sw} treatments.

349

350 ^{11}B NMR studies by Mavromatis et al. (2015) and Noireaux et al. (2015) have recently shown
351 that inorganically precipitated calcite contains up to 65% trigonal boron, although a linear
352 relationship between pH_{sw} , and measured $\delta^{11}\text{B}$ of the calcite was maintained. However, these
353 studies did identify a significant relationship between the percentage of trigonal boron in the
354 lattice and calcite growth rate. Noireaux et al. (2015) observed that slow growth rate led to a
355 higher percentage of trigonal boron in the calcite lattice, and suggested that this indicates an
356 increase in boric acid incorporation (see also Mavromatis et al., 2015). The slowest growth rates
357 in our cultured *Neogoniolithon* sp. are found at pH_{sw} 8.19 and 7.49, where in contrast, our boron
358 isotope data suggests the smallest boric acid incorporation (Table 2). In light of these findings, it
359 seems unlikely that boric acid incorporation is a dominant driver of the heavy $\delta^{11}\text{B}$ (relative to
360 $\delta^{11}\text{B}$ of aqueous borate expected at that pH_{sw}) observed in cultured *Neogoniolithon* sp., or has a
361 significant influence on the relationship between skeletal $\delta^{11}\text{B}$ and pH_{sw} in this species.
362 Furthermore, although ^{11}B NMR studies may reveal that trigonal boron is present in the calcite
363 lattice, this may be a result of geometry change of the borate molecule during incorporation into
364 the calcite lattice, rather than direct incorporation of boric acid (Balan et al., 2016).

365

366 4.1.2 Rayleigh fractionation

367 Coralline algae calcification occurs intercellularly within the cell walls of the algae, which are
368 semi-isolated from seawater by adjacent cells. Nevertheless, these extracellular restricted
369 environments are likely to be permeable to seawater and maintained at elevated pH and calcite
370 saturation state to promote calcification. Rayleigh fractionation describes the process by which
371 molecules or ions are continuously removed from a closed or semi-closed system, leading to
372 progressive change in the elemental and/or isotopic composition of the residual fluid. The
373 precipitation of CaCO_3 in this semi-isolated calcification space may therefore lead to changes in
374 the elemental and isotopic composition of the algal calcite (as proposed for corals by Gaetani
375 and Cohen, 2006; Gagnon et al., 2007). For example, assuming that borate (isotopically lighter

376 than total seawater boron) is solely incorporated into coralline algal calcite, the remaining fluid
377 would become enriched in ^{11}B , imposing a heavier $\delta^{11}\text{B}$ composition on the later forming calcite.
378

379 The partition coefficient (K_D) of boron into calcite is described by equation (5).

$$380 \quad K_D = \frac{[B/Ca]_{CaCO_3}}{[B/Ca]_{seawater}} \quad (5)$$

381 There are several estimates for the K_D of boron, and all are much less than one (*ca.* 0.0005; Yu et
382 al., 2007; Stoll et al., 2012). Consequently, as calcification progresses, Rayleigh fractionation
383 drives an increase in the B/Ca ratio of the residual fluid, thereby increasing B/Ca of the latterly
384 precipitated CaCO_3 . In theory, therefore, Rayleigh fractionation may be sufficient to describe
385 both the observed enrichment in ^{11}B in coralline algae calcite relative to seawater borate
386 (Figure 2), the observed relationships between pH_{sw} and both coralline algal B/Ca (Figure 5)
387 and $\delta^{11}\text{B}$ (Figure 2), as well as the observed correlation between coralline algal B/Ca and $\delta^{11}\text{B}$
388 (Figure 3).

389

390 However, the study of boron incorporation into deep sea scleractinian corals by Stewart et al.
391 (2016) shows that Rayleigh fractionation is unable to drive significant changes in skeletal $\delta^{11}\text{B}$
392 and B/Ca from unmodified seawater (*i.e.* [B] of $432 \mu\text{mol kg}^{-1}$; [Ca] of $10.3 \text{ mmol kg}^{-1}$; salinity 35
393 psu) given a typical biogenic carbonate B/Ca of $\sim 600 \mu\text{mol mol}^{-1}$ because insufficient borate is
394 removed at each incremental step of precipitation to drive the observed change in CaCO_3 $\delta^{11}\text{B}$.
395 Thus Rayleigh fractionation can only explain the relationship observed in Figure 3 between
396 B/Ca and $\delta^{11}\text{B}$ if the B/Ca ratio of the calcifying fluid is very much reduced relative to that of
397 seawater and the partition coefficient is higher than estimates from inorganic experiments (in
398 order to maintain the observed B/Ca ratio). For instance, a Rayleigh model fitted to the $\delta^{11}\text{B}$ and
399 B/Ca data in this study suggests a high K_D of 0.5, and a 98.5% reduction in seawater boron
400 content at the site of calcification. While this is a possibility in coralline algae as calcification
401 occurs within a semi-restricted space, the inverse correlation between Sr/Ca and B/Ca when
402 both elements have a K_D of <1 within calcite (defined in equation 5; Figure 5), suggests that

403 Rayleigh fractionation is unlikely to account for the entirety of the observed ^{11}B enrichment in
404 *Neogoniolithon* sp. relative to seawater borate, as well as the observed relationships between
405 pH_{sw} and $\delta^{11}\text{B}$, and B/Ca.

406

407 *4.2 Calcification rate and implications for coralline red algae in a high- CO_2 world*

408 Boron isotope characteristics of *Neogoniolithon* coralline red algae are unlikely to result from
409 boric acid incorporation or Rayleigh fractionation. Recent inorganic precipitation experiments
410 have highlighted the importance of calcification rate in controlling B/Ca in calcite (Gabitov et al.,
411 2014; Mavromatis et al., 2015; Noireaux et al., 2015; Uchikawa et al., 2015). Here we find strong
412 correlation between calcification rate and B/Ca ($R^2 = 0.40$, $p < 0.01$), which is therefore entirely
413 consistent with pH_{cf} elevation increasing Ω and borate concentration at the site of calcification,
414 thereby driving increased boron incorporation into the algal calcite. Although this might be
415 expected to also increase Sr/Ca given inorganic experiments (*e.g.* Böhm et al., 2012), the Sr/Ca
416 in the cultured coralline algae exhibits a positive correlation with DIC ($\mu\text{mol kgsw}^{-1}$; Figure
417 SM1A); a relationship recently documented in foraminifera (Keul et al., 2017). This points
418 towards a new proxy in coralline algae that has potential to fully resolve the carbonate system.

419

420 We are then left with the possibility that $\delta^{11}\text{B}$ of the algal calcite reflects pH_{cf} pursuant to the
421 $\delta^{11}\text{B}$ - pH_{sw} relationship, as proposed for scleractinian corals (*e.g.* McCulloch et al., 2012b). Since
422 pH_{cf} will largely control calcite saturation state (Ω) at the site of calcification, calcification rate
423 should exhibit a strong relationship with $\delta^{11}\text{B}$ and pH_{cf} . This is apparent when calcification rates
424 of individual algal specimens (Ries et al., 2009) are plotted against their respective $\delta^{11}\text{B}$ -derived
425 values of pH_{cf} (Figure 6A). The observed relationship between coralline algal calcification rate
426 and pH_{sw} (Figure 6B; *i.e.*, increased calcification under slightly elevated $p\text{CO}_2$, reduced
427 calcification at extremely elevated $p\text{CO}_2$; Ries et al., 2009) may thus arise from the relationship
428 between pH_{sw} and pH_{cf} (Figure 4). Furthermore, the ability of *Neogoniolithon* algae to raise pH_{cf}
429 relative to pH_{sw} increases under more acidified conditions, with ΔpH increasing from 0.85 (\pm

430 0.11) to 1.26 (± 0.22) between pH_{sw} of 8.19 to 7.91. These results are consistent with three
431 coralline algae species (including a crustose *Neogoniolithon* sp.) cultured at variable pH_{sw} by
432 Cornwall et al. (2017), which also exhibit a similar increase in ΔpH from ca. 0.8 to ca. 1.1
433 between pH_{sw} of 8.08 to 7.64 (Cornwall et al., 2017). However, our observation that ΔpH
434 levelled off under the two most acidic treatments suggests that there is a limit to the extent to
435 which the branching species of *Neogoniolithon* can elevate pH_{cf} relative to pH_{sw} . This limit may
436 also exist for those species examined by Cornwall et al. (2017) but is not resolvable because
437 their pH_{cf} data are confined to a narrower pH_{sw} range, with only three pH_{sw} treatments
438 examined that fall within the linear portion of our pH_{cf} vs. pH_{sw} relationship. Nonetheless, taken
439 together, our study and that of Cornwall et al. (2017) illustrate that pH_{cf} in coralline algal
440 therefore appears to promote calcification in moderately acidified seawater (down to pH_{sw}
441 7.95), which is most likely due to CO_2 -fertilisation of photosynthesis. This supports the previous
442 observation that photosynthesis in some marine algae is CO_2 -limited up to ca. $1000 \mu\text{atm } p\text{CO}_2$
443 (Bowes, 1993). Our new data at low pH_{sw} , however, reveals that no additional benefit for
444 photosynthesis in this coralline alga appears to be conferred by increasing $p\text{CO}_2$ from 903 to
445 $2856 \mu\text{atm } p\text{CO}_2$, while the accompanying increase in acidity and resulting decrease in Ω of the
446 culture solution has a clear detrimental effect on the calcification rate of the algae.

447

448 As has been demonstrated for scleractinian corals (McCulloch et al., 2012b), the ability of
449 coralline algae to elevate pH_{cf} may confer resilience to the deleterious effects of ocean
450 acidification, thereby giving them an advantage over calcifying taxa competing for space on the
451 seafloor that lack this ability. Specifically, our data suggest that species-specific pH_{sw} optima
452 exist at pH_{sw} ca. 8 for maximising both pH_{cf} and calcification rates of *Neogoniolithon* sp.
453 However, that pH_{cf} and algal calcification rates begin to dramatically decline as pH_{sw} is
454 decreased from 7.9 to 7.5 indicates that there are limits to the extent that coralline algae can
455 mitigate the effects of more extreme ocean acidification. Indeed, at extremely low pH_{sw} ,
456 mineralogical changes (high Mg calcite to gypsum ratio) are induced in other species of coralline

457 algae (Kamenos et al., 2016). Together, these findings have implications for how *Neogoniolithon*
458 sp. will cope with increasing ocean acidification in the future.

459

460 *4.3 Intra-treatment variability and implications for the boron isotope proxy*

461 One notable feature of the $\delta^{11}\text{B}$ presented here for *Neogoniolithon* sp. is the degree of variability
462 between specimen replicates within pH_{sw} treatments. Although some degree of scatter between
463 replicates is often observed in other culture studies, in this case it reached ca. 6 ‰. Some of this
464 scatter may be influenced by the heterogeneity of the bulk samples, as microstructural
465 differences have been shown to affect $\delta^{11}\text{B}$ in aragonitic corals by up to 10 ‰ (Blamart et al.,
466 2007), and laser ablation $\delta^{11}\text{B}$ has revealed variations of up to 6 ‰ in other species of coralline
467 algae (Fietzke et al., 2015). That the spread in $\delta^{11}\text{B}$ is still fairly large at the pH_{sw} closest to
468 ambient confirms this is not a methodological artefact where pre-experimental skeleton is
469 inadvertently sampled, but rather is a primary feature of this species of coralline red algae. This
470 is also confirmed by the lack of correlation between the scatter from the mean $\delta^{11}\text{B}$ for each
471 treatment and the mass of CaCO_3 measured (Figure SM2).

472

473 Despite this spread in $\delta^{11}\text{B}$ for a given treatment, there remains good correlation between B/Ca
474 and $\delta^{11}\text{B}$ (Figure 3). Although the strength of this correlation is perhaps unexpected given some
475 related studies (e.g. Douville et al., 2010; Henehan et al., 2015), this further supports the
476 assertion that coralline algal calcification rate, $\delta^{11}\text{B}$ and B/Ca are controlled by pH_{cf} of the algae,
477 and that there is considerable variability in pH_{cf} amongst individuals.

478

479 The finding that coralline red algal $\delta^{11}\text{B}$ responds to pH_{sw} suggests that this is a potential taxon
480 for reconstructing palaeo- pH_{sw} ; a conclusion that is particularly noteworthy given coralline
481 algae's ability to produce long growth records in high-latitude oceans, where palaeo- pH_{sw}
482 records are sparse (Fietzke et al., 2015). Despite these encouraging results, further work on
483 *Neogoniolithon* is clearly required to determine whether the $\delta^{11}\text{B}$ of this genus of coralline algae

484 offers the precision and accuracy needed to reliably reconstruct past changes in pH_{sw} ,
485 particularly in light of their strong inter-specimen variability in boron geochemistry.

486

487 *5.0 Conclusion*

488 We find that statistically significant relationships exist in cultures of the coralline red algae
489 *Neogoniolithon* sp. between $\delta^{11}\text{B}$ and pH_{sw} , $\delta^{11}\text{B}$ and skeletal B/Ca, and pH_{cf} and net calcification
490 rate. Skeletal $\delta^{11}\text{B}$ in this species is considerably elevated compared to $\delta^{11}\text{B}$ of both seawater
491 borate and most other examples of biogenic carbonate, suggesting an average pH_{cf} increase of
492 more than 1 pH unit relative to pH_{sw} . An observed correlation between calcification rate and
493 pH_{cf} suggests that the algae promote calcification by elevating pH_{cf} . Furthermore, the
494 observation that ΔpH increased as pH_{sw} decreased from 8.2 to 7.9 suggests that this species of
495 coralline red algae is able to mitigate the effects of moderate ocean acidification *via* pH
496 regulation at the site of calcification. However, the observation that pH_{cf} and calcification rates
497 decreased when pH_{sw} was reduced to 7.5 suggest that there is a limit to the extent to which this
498 species can mitigate the effects of extreme ocean acidification.

499

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741 *Figure captions*

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743 **Figure 1. Calcification rates of coralline red algae plotted against pH_{sw} .** Comparison between
744 calcification trends in coralline red algae described in Smith and Roth (1979; *Bossiella orbigniana*) (A) and
745 Ries *et al.* (2009; *Neogoniolithon* sp.) (B). Although calcification rates are reported in different units, both
746 studies suggest that coralline algae exhibit a parabolic calcification response to CO₂-induced ocean
747 acidification, with an optimum near pH_{sw} 7.9.

748 **Figure 2. $\delta^{11}B$ of the coralline red algae *Neogoniolithon* sp. measured using MC-ICPMS, plotted**
749 **against pH_{sw} .** Black circles filled with grey represent replicates measured at each pH_{sw} , and the mean
750 $\delta^{11}B$ of each culture experiment is shown as a filled black circle. The $\delta^{11}B$ of all measured samples are
751 elevated relative to aqueous borate (blue line) by +12 ‰ on average. $\delta^{11}B$ compositions of a crustose
752 species of *Neogoniolithon* coralline red alga and various scleractinian corals grown at different pH_{sw} are
753 plotted in open coloured symbols (Cornwall *et al.*, 2017; Honisch *et al.*, 2004; Reynaud *et al.*, 2004; Krief
754 *et al.*, 2010; Anagnostou *et al.*, 2012; McCulloch *et al.*, 2012a; Holcomb *et al.*, 2014).

755 **Figure 3. Least squares linear regression of B/Ca and Sr/Ca against $\delta^{11}B$ composition.** (A) B/Ca
756 ratios in *Neogoniolithon* sp. show a strong positive correlation when regressed against $\delta^{11}B$ composition.
757 (B) Sr/Ca ratios show slightly less well-defined trends when regressed against $\delta^{11}B$ composition,
758 although both reveal statistically significant correlations.

759 **Figure 4. pH_{cf} vs. pH_{sw} (A) and ΔpH ($pH_{cf} - pH_{sw}$) vs. pH_{sw} (B).** An apparent parabolic relationship is
760 observed in A, with a maximum at $pH_{cf} \sim 7.95$. In B, ΔpH also exhibits a similar relationship with pH_{sw}
761 suggesting that coralline red algae increase their pH_{cf} by increasingly larger amounts under acidified
762 conditions to support biogenic calcification. At extremely low pH_{sw} , the shape of the curve suggests that
763 coralline red algae have reached the limit of the extent to which they can elevate pH_{cf} relative to pH_{sw} . The
764 filled black circles indicate mean values. This branching species of *Neogoniolithon* coralline red algae is
765 compared with a crustose species of *Neogoniolithon* (stars) from Cornwall *et al.* (2017).

766 **Figure 5. B/Ca and Sr/Ca regressed against pH_{sw} .** Sr/Ca ratios (A) of *Neogoniolithon* sp. are strongly
767 linearly correlated with pH_{sw} , while B/Ca is strongly correlated with an apparent parabolic relationship
768 with pH_{sw} (B). Sr/Ca (C) is not significantly correlated with B/Ca, although the trend is nearly significant.
769 The filled black circles indicate mean values.

770 **Figure 6. The relationship between the pH_{cf} and net calcification of *Neogoniolithon* sp.** (A) This
771 positive correlation between the mean pH_{cf} and mean calcification rate indicates a reduction in
772 calcification rate with decreasing pH_{cf} . (B) Across treatments, pH_{cf} (black circles) is influenced by pH_{sw} ,
773 which also affects net calcification (red squares). The similarity between the two negative curves
774 highlights the link between calcification rate and pH_{sw} , but also reveals the resilience of coralline red
775 algae to moderate ocean acidification. The algae are able to mitigate moderate pH_{sw} reduction, but are
776 unable to calcify efficiently at extremely low pH_{sw} values.

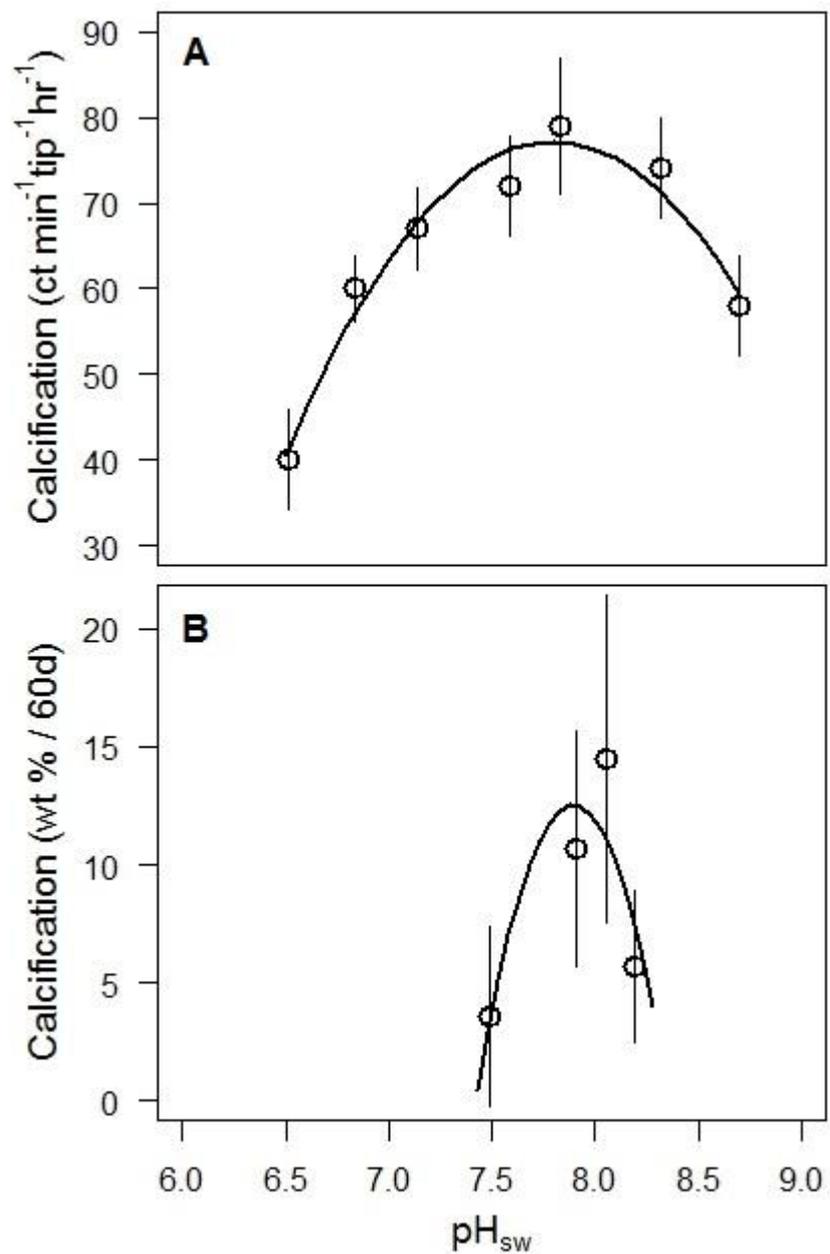
777 *Table captions*

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779 **Table 1. Summary of each experimental treatment showing measured element ratios and $\delta^{11}B$**
780 **composition.** The mean of each variable measured is shown in bold, 1σ are shown in parentheses. The
781 sample shown in red is anomalous and is therefore excluded from subsequent discussion (also excluded
782 from means). Therefore $n = 16$ for all regression analyses.

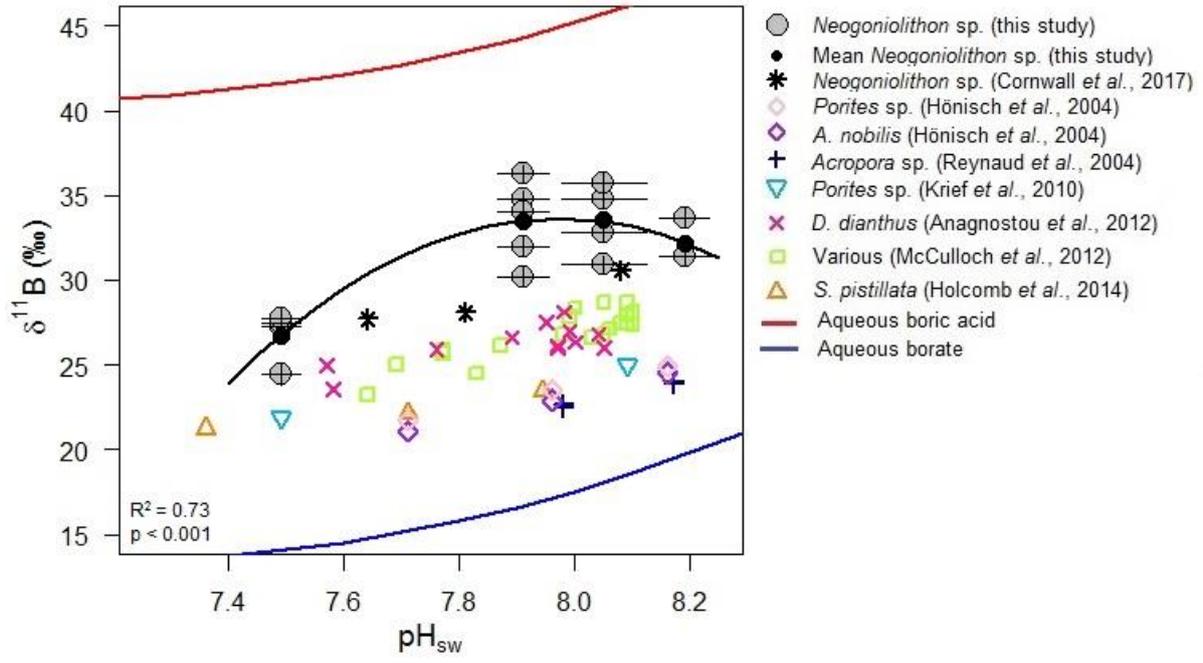
783 **Table 2. Mean skeletal $\delta^{11}B$ compositions for each experimental treatment, along with percentage**
784 **of boric acid (enriched in ^{11}B by 27.2 ‰) required to be incorporated into the algal calcite to**
785 **generate the measured $\delta^{11}B$ composition, assuming that $\delta^{11}B$ of the borate portion of the algal**
786 **calcite is equal to seawater borate.** Net calcification indicates that algae from the pH_{sw} 8.19 and 7.49
787 treatments have the slowest calcification rates, yet also require the smallest apparent proportion of boric
788 acid. pH_{cf} indicates mean calcification site pH for each treatment, and ΔpH describes the change in pH
789 according to the equation $\Delta pH = pH_{cf} - pH_{sw}$. 1σ are shown in parentheses.

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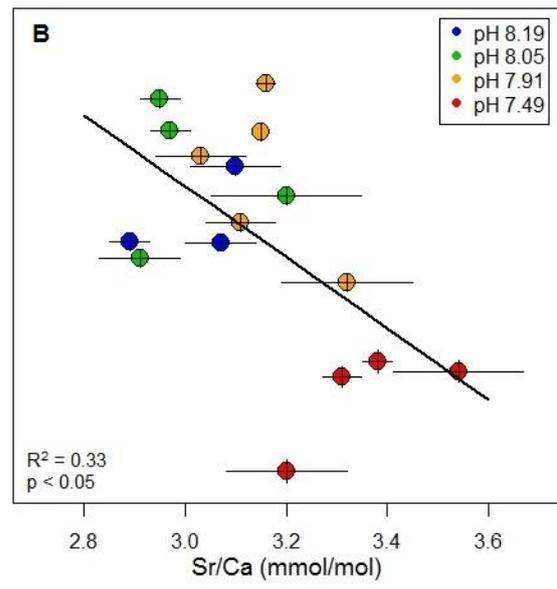
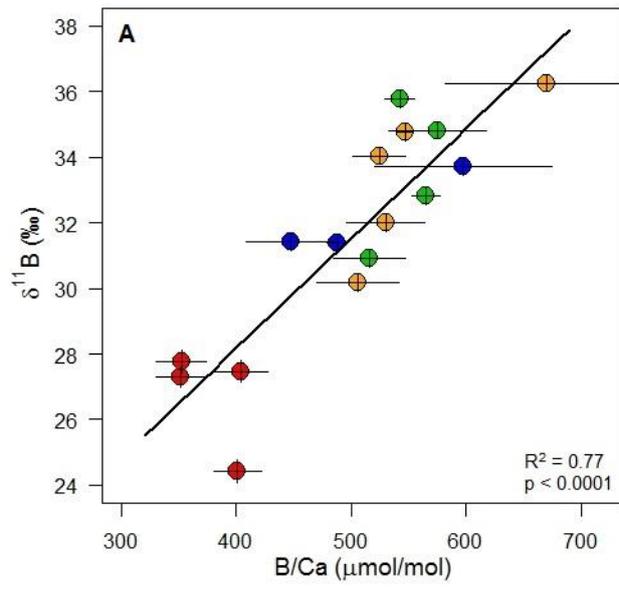
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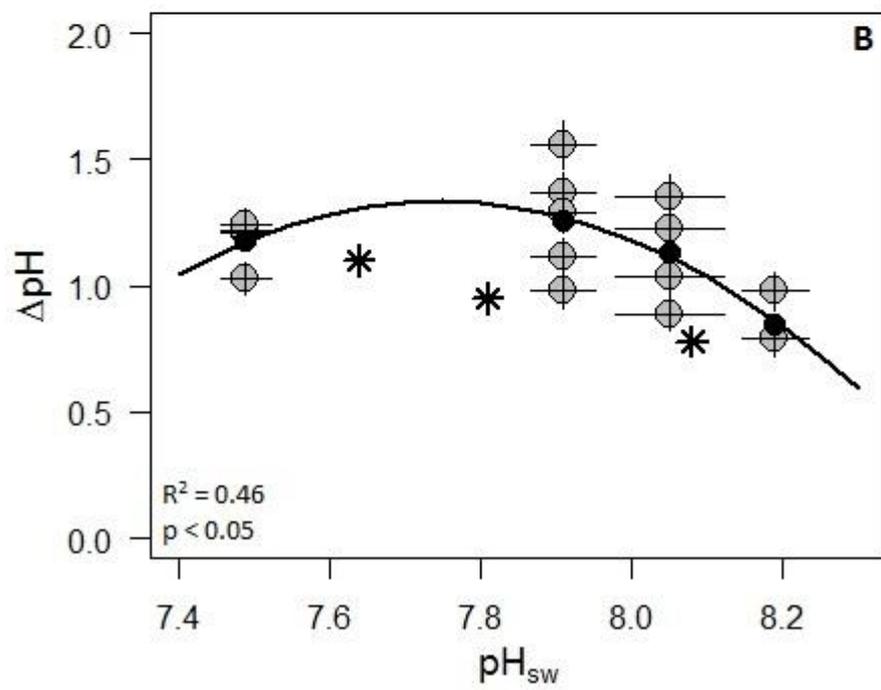
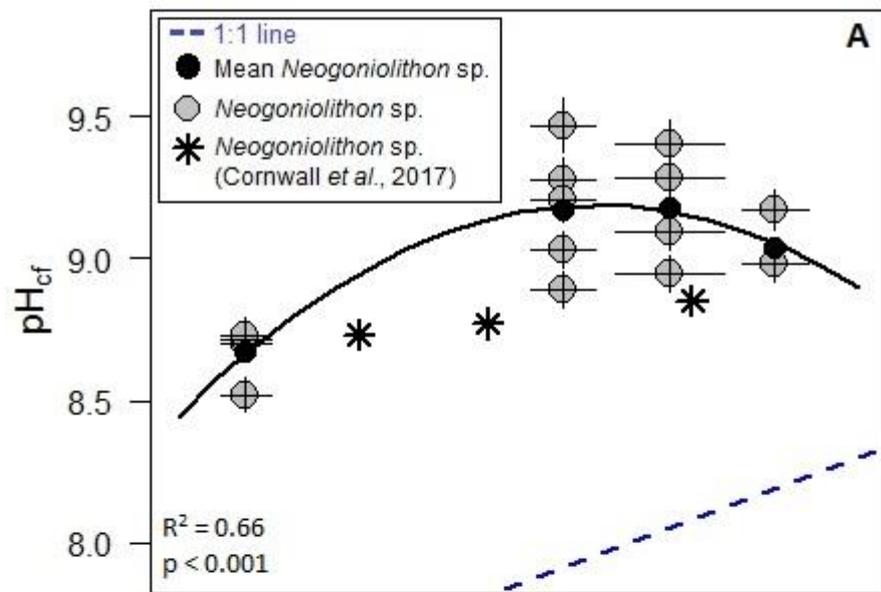
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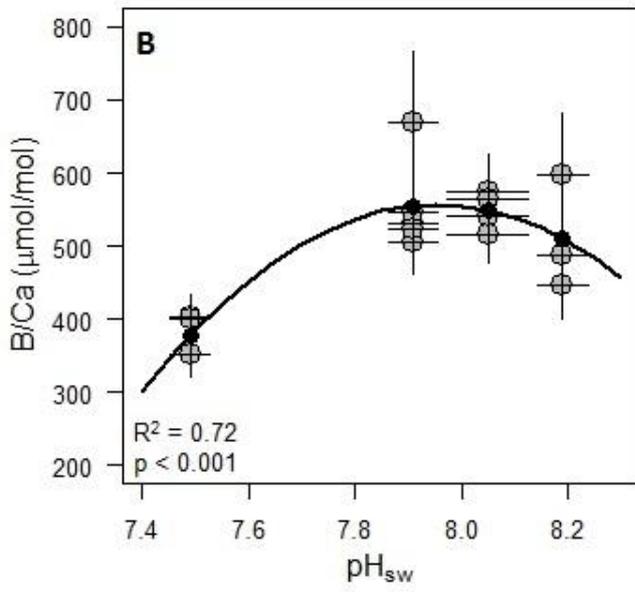
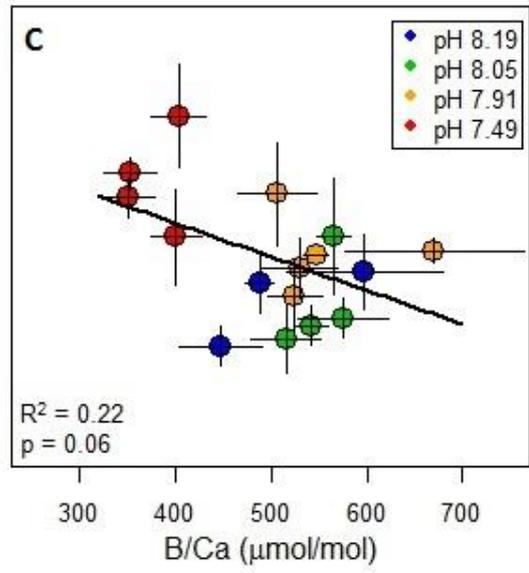
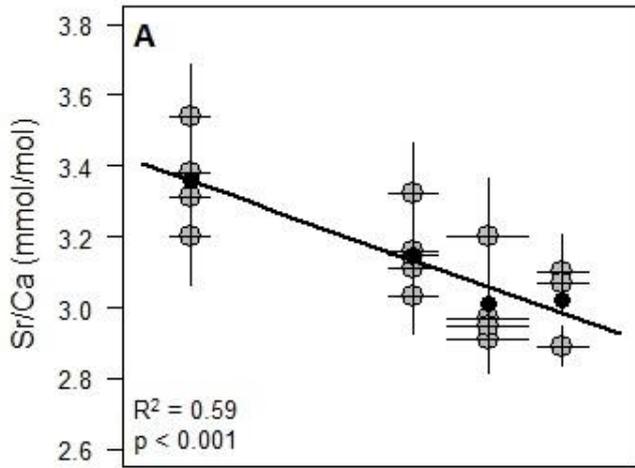
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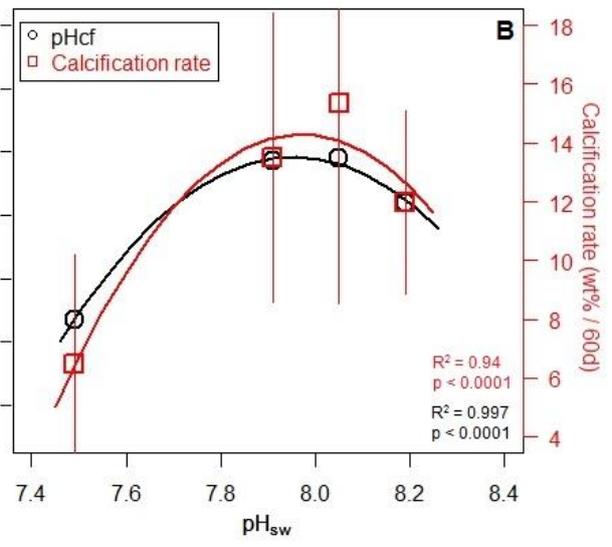
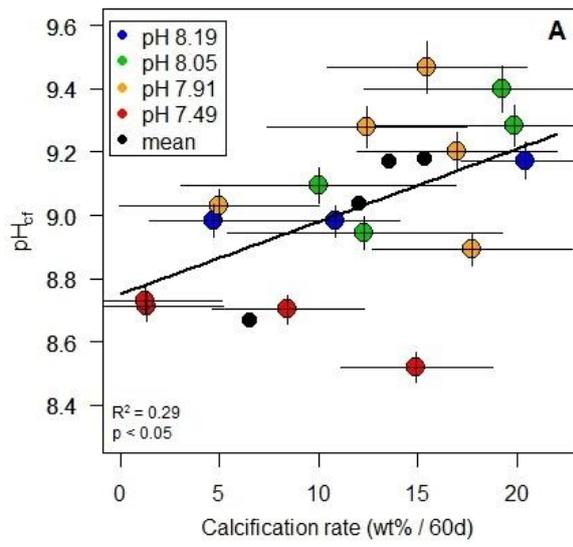
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Table 1.

$p\text{CO}_2$	pH	$\delta^{11}\text{B} \text{‰}$				$\text{B}/\text{Ca} \mu\text{mol mol}^{-1}$				$^{11}\text{B} \text{ nmol}$				$\text{Sr}/\text{Ca} \text{ nmol}$	
		Replicate	Duplicate	Average	Experiment mean	Replicate	Duplicate	Average	Experiment mean	Replicate	Duplicate	Average	Experiment mean	Replicate	Duplicate
409 (6)	8.19 (0.03)	40.61 (0.10)	39.18 (0.11)	39.90	32.17 (1.33)	815 (41)	686 (34)	751	511 (78)	800 (132)	533 (57)	667	415 (62)	2.89 (0.14)	2.99 (0.15)
		31.44 (0.13)	31.38 (0.11)	31.41		454 (29)	439 (39)	447		354 (84)	415 (40)	395		2.85 (0.10)	2.93 (0.04)
		33.70 (0.11)				598 (73)				590 (83)				3.10 (0.08)	
		31.40 (0.12)				488 (5)				530 (41)				3.07 (0.06)	
606 (7)	8.05 (0.06)	35.02 (0.10)	34.60 (0.11)	34.81	33.58 (2.16)	628 (52)	521 (24)	575	550 (26)	545 (24)	440 (50)	493	516 (36)	2.99 (0.01)	2.95 (0.04)
		30.90 (0.10)				516 (27)				466 (32)				2.91 (0.07)	
		32.83 (0.14)				565 (8)				500 (8)				3.20 (0.14)	
		35.77 (0.11)				542 (9)				603 (65)				2.95 (0.03)	
903 (12)	7.91 (0.03)	32.05 (0.16)	31.99 (0.10)	32.02	33.45 (2.38)	488 (44)	572 (15)	530	555 (66)	452 (18)	513 (25)	483	477 (49)	3.02 (0.09)	3.19 (0.03)
		34.75 (0.11)				547 (3)				371 (75)				3.15 (0.00)	
		30.18 (0.15)				506 (32)				488 (7)				3.32 (0.12)	
		36.26 (0.10)				670 (84)				641 (115)				3.16 (0.01)	
		34.03 (0.10)				524 (19)				401 (54)				3.03 (0.08)	
2856 (54)	7.49 (0.02)	27.31 (0.18)			26.75 (1.56)	352 (18)			377 (29)	69 (66)			163 (74)	3.31 (0.03)	
		27.47 (0.18)				404 (19)				373 (149)				3.54 (0.13)	
		27.78 (0.21)				352 (18)				100 (44)				3.38 (0.02)	
		24.42 (0.22)				401 (17)				108 (39)				3.20 (0.11)	

804

Table 2.

$p\text{CO}_2$ μatm	pH_{sw}	$\delta^{11}\text{B}$ ‰	$\text{B}(\text{OH})_3$ %	Net calcification wt% / 60 days	pH_{cr}	ΔpH
409 (6)	8.19 (0.03)	32.17 (1.33)	44 (4)	5.7 (2.9)	9.04 (0.11)	0.85 (0.11)
606 (7)	8.05 (0.06)	33.58 (2.16)	57 (7)	14.5 (6.6)	9.18 (0.20)	1.13 (0.20)
903 (12)	7.91 (0.03)	33.45 (2.38)	60 (8)	10.7 (4.7)	9.17 (0.22)	1.26 (0.22)
2856 (54)	7.49 (0.02)	26.75 (1.56)	46 (6)	3.6 (3.5)	8.67 (0.10)	1.18 (0.10)

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806

807 *Supplementary material*

808 *Table captions*

809 **Table SM1. Further details of the culture experiment from Ries et al. (2009).** Values for all tanks as
810 well as individual culture treatment tanks are shown with $\pm 1\sigma$.

811 **Table SM2. Statistical analysis of all parameters investigated in this study.** Regressions, regression
812 equations and significance values are all described in further detail.

813 *Figure captions*

814 **Figure SM1. Sr/Ca vs. DIC (A), B/Ca vs. DIC (B), and B/Ca vs. net calcification (C).** These relationships
815 were explored further following a recent paper investigating foraminiferal Sr/Ca as a new carbonate
816 system proxy (Keul et al., 2017). With an enhanced DIC influx, Ω increases, and therefore Ca influx
817 decreases, hence a positive relationship can be found between DIC and Sr/Ca. Net calcification appears to
818 have a dominant role in determining B/Ca, as opposed to DIC determining Sr/Ca. B/Ca ratios have a
819 strong positive correlation with net calcification, and whilst there is a negative relationship present
820 between B/Ca and DIC, this is most likely due to typically lower calcification rates at higher DIC.

821 **Figure SM2. Size of sample plotted against difference from mean $\delta^{11}\text{B}$.** This relationship reveals there
822 are comparable ranges of difference between the measured $\delta^{11}\text{B}$ and the mean $\delta^{11}\text{B}$ for every pH_{sw}
823 treatment, no matter the size of sample measured. This indicates there is no bias towards larger samples,
824 and that $\delta^{11}\text{B}$ is unaffected by the size of the initial sample.

825 *References*

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827 one atmosphere total pressure. *Am. J. Sci.* **283**, 780–799.

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829 ocean acidification. *Geology* **37**, 1131–1134.

830 Roy R. N., Roy L. N., Vogel K. M., Porter Moore C., Pearson T., Good C. E., Millero F. J. and Campbell D. M.
831 (1993) The dissociation constants of carbonic acid in seawater at salinities 5 to 45 and temperatures 0
832 degrees C to 45 degrees C. *Mar. Chem.* **44**, 249–267.

833

Table SM1.

	All	pH 8.19	pH 8.05	pH 7.91	pH 7.49
Sample location	Atlantic Ocean, FL				
Number of tanks	4				
Filter rate (L/h)	600				
Irradiance (W/m ²)	426				
K_{sp}	(Mucci, 1983)				
K_1 and K_2	(Roy et al., 1993)				
Water temperature (°C)		25.0 ± 0.055	25.0 ± 0.152	25.1 ± 0.164	24.9 ± 0.130
Salinity (psu)		31.8 ± 0.207	31.7 ± 0.118	31.5 ± 0.155	31.8 ± 0.258
Ω_{arag}		3.12 ± 0.221	2.40 ± 0.420	1.84 ± 0.129	0.90 ± 0.050
DIC		1738 ± 50.35	1786 ± 100.71	1903 ± 45.91	2350 ± 33.21

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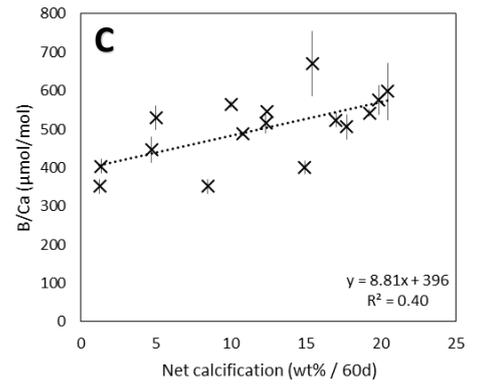
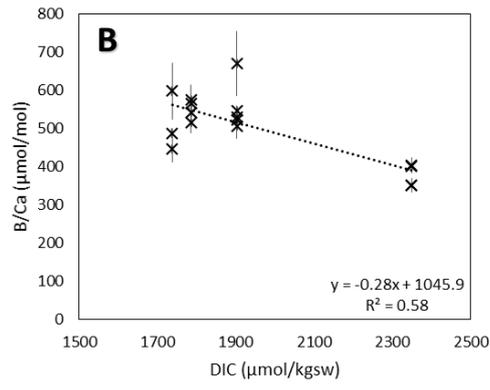
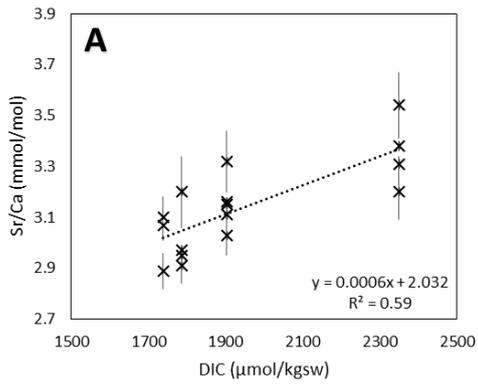
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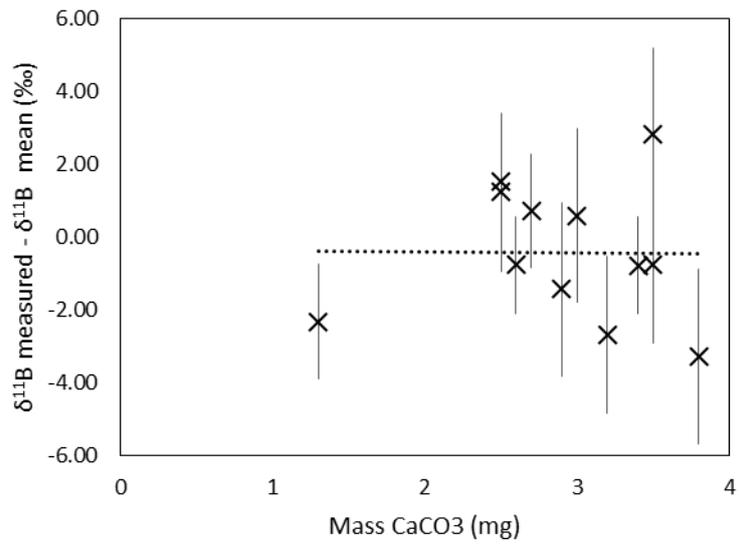
Table SM2.

Parameter 1	Parameter 2	Regression	Equation	R²	p
$\delta^{11}\text{B}$	pH _{sw}	Curve	$y = -30x^2 + 472x - 1846$	0.73	< 0.001
$\delta^{11}\text{B}$	B/Ca	Linear	$y = 0.034x + 14.8$	0.77	< 0.0001
$\delta^{11}\text{B}$	Sr/Ca	Linear	$y = -10.8x + 65.6$	0.33	< 0.05
pH _{cf}	pH _{sw}	Curve	$y = -2.4x^2 + 38.5x - 143.8$	0.66	< 0.001
ΔpH	pH _{sw}	Curve	$y = -2.4x^2 + 37.6x - 144.3$	0.46	< 0.05
Sr/Ca	pH _{sw}	Linear	$y = -0.53x + 7.3$	0.59	< 0.001
B/Ca	pH _{sw}	Curve	$y = -833x^2 + 13258x - 52173$	0.72	< 0.001
Sr/Ca	B/Ca	Linear	$y = -0.001x + 3.6$	0.22	0.06
pH _{cf}	Calcification rate	Linear	$y = 0.023x + 8.8$	0.29	< 0.05
Calcification rate	pH _{sw}	Curve	$y = -34x^2 + 545x - 2161$	0.94	< 0.0001

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