

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 (CO<sub>2</sub>) 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<sub>sw</sub>) 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<sub>cf</sub>) in scleractinian corals, providing mechanistic insights  
17 into coral biomineralisation and the impact of variable pH<sub>sw</sub> 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<sub>cf</sub>, and investigates how this taxon's pH<sub>cf</sub> 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 pCO<sub>2</sub> scenarios – averaging ( $\pm 1\sigma$ ) 409 ( $\pm 6$ ), 606 ( $\pm 7$ ), 903 ( $\pm 12$ ) and 2856 ( $\pm 54$ )  
22  $\mu\text{atm}$ , corresponding to average pH<sub>sw</sub> ( $\pm 1\sigma$ ) of 8.19 ( $\pm 0.03$ ), 8.05 ( $\pm 0.06$ ), 7.91 ( $\pm 0.03$ ) and  
23 7.49 ( $\pm 0.02$ ) 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<sub>sw</sub> treatments by up to 18 ‰. Although substantial variability in  $\delta^{11}\text{B}$   
25 exists between replicate samples cultured at a given pH<sub>sw</sub> (smallest range = 2.32 ‰ at pH<sub>sw</sub>  
26 8.19, largest range = 6.08 ‰ at pH<sub>sw</sub> 7.91), strong correlations are identified between  $\delta^{11}\text{B}$  and  
27 pH<sub>sw</sub> ( $R^2 = 0.72$ ,  $p < 0.0001$ ,  $n = 16$ ) 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<sub>cf</sub> as previously observed for scleractinian corals, the  
29 average pH<sub>cf</sub> across all experiments was 1.20 pH units (0.79 to 1.56) higher than pH<sub>sw</sub>, with the

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

37

### 38 *1.0 Introduction*

39 Atmospheric  $\text{CO}_2$  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 ( $\text{pH}_{\text{sw}}$ ) 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  $\text{pH}_{\text{sw}}$  of ca. 7.8, potentially reaching even lower levels at high latitudes. This large and  
45 rapid reduction in global  $\text{pH}_{\text{sw}}$  will result in an environment that is potentially challenging to  
46 marine organisms that rely on biogenically produced  $\text{CaCO}_3$  (Doney et al., 2009).

47

48 Ocean acidification affects biogenic calcification by reducing the  $\text{CaCO}_3$  saturation state of  
49 seawater ( $\Omega = [\text{Ca}^{2+}][\text{CO}_3^{3-}]/K_{\text{sp}}^*$ ; where  $K_{\text{sp}}^*$  is the stoichiometric solubility product of  $\text{CaCO}_3$  at  
50 in situ conditions of temperature, salinity and pressure). Reductions in  $\Omega$  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  $\text{CO}_2$  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 CaCO<sub>3</sub> 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 % MgCO<sub>3</sub>), 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 CO<sub>2</sub> 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 calciblastic  
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  $\text{pH}_{\text{sw}}$ , suggesting that the algal calcification increases in response to moderate  
86 elevations in  $\text{pCO}_2$ , but decreases in response to extreme increases. However, Ries et al. (2009)  
87 observed maximum calcification between  $\text{pH}_{\text{sw}}$  7.9 and 8.1 ( $\Omega_{\text{A}} \sim 2.0$  to 2.3; where  $\Omega_{\text{A}}$  is the  
88 saturation state for the aragonite  $\text{CaCO}_3$  polymorph), while Smith and Roth (1979) observed  
89 maximum calcification between  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$ .

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  $\text{pH}_{\text{sw}}$ . This mitigation  
97 of extreme ocean acidification has been shown to translate into coralline algae survival in low  
98  $\text{pH}_{\text{sw}}$  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  $\text{CO}_2$ -limited conditions. Experimental work has shown that  
103 photosynthesis in some marine algae is  $\text{CO}_2$ -limited up to *ca.* 1000  $\mu\text{atm}$   $\text{pCO}_2$  (Bowes, 1993).  
104 Therefore, the additional energy from photosynthesis as  $\text{pCO}_2$  becomes elevated up to *ca.* 1000  
105  $\mu\text{atm}$  may stimulate calcification within calcifying marine algae, despite the associated decrease  
106 in  $\text{pH}_{\text{sw}}$ . 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  $\text{CO}_2$  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  $\text{CO}_2$  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  $\text{pH}_{\text{sw}}$  and  $\text{CaCO}_3$  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 ( $\text{pH}_{\text{cf}}$ ), 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  $\text{pH}_{\text{cf}}$  that is significantly higher than measured ambient  $\text{pH}_{\text{sw}}$  (by  
123 ca. 0.6 pH units;  $\Delta\text{pH} = \text{pH}_{\text{cf}} - \text{pH}_{\text{sw}}$ ), suggesting an increase of  $\text{pH}_{\text{cf}}$  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  $\text{pH}_{\text{sw}}$  conditions that  
126 demonstrate the response of  $\text{pH}_{\text{cf}}$  to changes in  $\text{pH}_{\text{sw}}$  are currently sparse (*e.g.* the only other  
127 such study is Cornwall et al., 2017). Here, the boron isotope approach to estimating  $\text{pH}_{\text{cf}}$  is  
128 applied to a branched *Neogoniolithon* sp. cultured under four  $\text{pCO}_2$  conditions that allow us to  
129 assess the potential impacts of ocean acidification on  $\text{pH}_{\text{cf}}$  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  $\text{pH}_{\text{sw}}$  (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  $39.61 \pm 0.04$  ‰  
 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  $\alpha_B$  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 ( $\pm 6$ ), 606 ( $\pm 7$ ), 903 ( $\pm 12$ ) and 2856 ( $\pm 54$ )  $\mu atm$ , resulting in  $pH_{sw}$  values ( $\pm 1\sigma$ ) of 8.19 ( $\pm 0.03$ ), 8.05 ( $\pm 0.06$ ), 7.91 ( $\pm 0.03$ ) and 7.49 ( $\pm 0.02$ ), respectively (Ries et al., 2009). The algae were grown for 60 days in 38 L aquaria in filtered Atlantic Ocean seawater (0.2  $\mu m$ ; Cape Cod, Massachusetts). The cultures were maintained at average aragonite saturation states ( $\pm 1\sigma$ ) of 3.12 ( $\pm 0.22$ ), 2.40 ( $\pm 0.42$ ), 1.84 ( $\pm 0.13$ ) and 0.90 ( $\pm 0.05$ ), 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  $\mu atm$ , and declining with an increase in  $pCO_2$  to 903 and 2856  $\mu atm$  (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  $\text{pH}_{\text{sw}}$  8.19, 8.05 and 7.49). Duplicate analyses were performed on all replicate  
191 specimens except those from the  $\text{pH}_{\text{sw}}$  7.49 treatment, due to the small mass of  $\text{CaCO}_3$   
192 mineralised under these high- $p\text{CO}_2$  conditions. Skeletal material produced exclusively under the  
193 experimental treatments was identified relative to a  $^{137}\text{Ba}$  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  $\mu\text{l}$  of an oxidative mixture of 10% hydrogen peroxide ( $\text{H}_2\text{O}_2$ )  
199 buffered with 0.1 M ammonium hydroxide ( $\text{NH}_4\text{OH}$ ). 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 ( $\text{HNO}_3$ ) and then dissolved in a minimal volume of 0.5 M  $\text{HNO}_3$ .

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  $\text{HNO}_3$ . 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  $1.4 \text{ ‰}$  compared with an average difference between other  
230 duplicate analyses of  $0.18 \text{ ‰}$ . 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  $2.3 \text{ ‰}$  at  $\text{pH}_{\text{sw}} 8.19$  to  $6.1 \text{ ‰}$  at  $\text{pH}_{\text{sw}} 7.91$ .  
234 The relationship between  $\delta^{11}\text{B}$  of *Neogoniolithon* sp. calcite and  $\text{pH}_{\text{sw}}$  (Figure 2) demonstrates  
235 that all  $\delta^{11}\text{B}$  measurements in this study lie considerably above the  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{cf}}$ , 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  $\text{pH}_{\text{sw}}$  vs.  $\delta^{11}\text{B}$  curve from the  $\text{pH}_{\text{sw}}$  vs. aqueous borate  $\delta^{11}\text{B}$  curve is generally  
244 consistent with the offset previously observed for corals grown at various  $\text{pH}_{\text{sw}}$  (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  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$  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),  $\text{pH}_{\text{cf}}$  calculated using  
260 equation 3 (assuming boron in the algal calcite is sourced solely from seawater borate) reveals  
261 an elevation of  $\text{pH}_{\text{cf}}$  relative to  $\text{pH}_{\text{sw}}$  by an average of 1.20 ( $\pm 0.22$ ) pH units (Figure 4A). There is  
262 a statistically significant linear positive correlation ( $R^2 = 0.45$ ,  $p < 0.01$ ) between  $\text{pH}_{\text{cf}}$  and  $\text{pH}_{\text{sw}}$ ,  
263 although once again a second-order polynomial model with an optimum near  $\text{pH}_{\text{sw}}$  7.95 better  
264 describes the data ( $R^2 = 0.66$ ,  $p < 0.001$ ). If this model of boron incorporation is correct,  $\Delta\text{pH}$   
265 plotted against  $\text{pH}_{\text{sw}}$  exhibits an apparent parabolic relationship with  $\text{pH}_{\text{sw}}$  ( $R^2 = 0.46$ ,  $p < 0.01$ ;  
266 Figure 4B).  $\Delta\text{pH}$  approaches a maximum mean of 1.26 pH units under the second most acidic  
267 treatment, and although these measurements fall within  $1\sigma$  of each mean, there is a significant  
268 reduction of  $\Delta\text{pH}$  at the most acidic treatment ( $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$  8.19,  
270 8.05 and 7.91 when compared to the algae cultured at  $\text{pH}_{\text{sw}}$  7.49, confirming that a reduction in  
271  $\text{pH}_{\text{sw}}$  causes a decrease in  $\text{pH}_{\text{cf}}$  of coralline red algae.

272

273 The B/Ca of the algal specimens range from 352 ( $\pm 18$ ) to 670 ( $\pm 84$ )  $\mu\text{mol mol}^{-1}$  (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.  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$  7.91  
279 to 52  $\mu\text{mol mol}^{-1}$  at  $\text{pH}_{\text{sw}}$  7.49.

280

281 Calcite Sr/Ca ranges from 2.85 ( $\pm 0.10$ ) to 3.54 ( $\pm 0.13$ )  $\text{mmol mol}^{-1}$  and exhibits a statistically  
282 significant negative linear correlation with  $\text{pH}_{\text{sw}}$  ( $R^2 = 0.59$ ,  $p < 0.001$ ; 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 calcoblastic 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

306 algae therefore requires some key assumptions, including in particular that the algal  
307 calcification fluid has a total  $\delta^{11}\text{B}$  and salinity similar to that of ambient seawater. Nonetheless,  
308 recent studies have shown that calcein, which cannot be transported across cellular  
309 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  $\text{pH}_{\text{sw}}$  indicate that boron  
305 systematics of coralline algae do vary with respect to  $\text{pH}_{\text{sw}}$ . As expected from the existing  
306 understanding of the proxy, cultures at lower  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$ .  
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.  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{cf}}$  to promote calcification. Indeed  $\text{pH}_{\text{cf}}$  has been shown to increase during seasonal  
312 variations in  $\Delta\text{pH}$  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  $\Delta\text{pH}$  of ca. 0.8 – 1.1 pH units,  
315 depending on  $\text{pH}_{\text{sw}}$ .

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  $\text{pH}_{\text{cf}}$ , and  
320 (3) only seawater borate is incorporated into the coralline algal skeleton, suggests that  
321 *Neogoniolithon* sp. undergoes a large  $\text{pH}_{\text{cf}}$  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 ( $\text{CO}_3^{2-}$ ) found in the algal calcite lattice and, whilst boric acid holds no charge, it  
331 may be incorporated as an impurity. Solid state  $^{11}\text{B}$  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  $^{11}\text{B}$   
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  $^{11}\text{B}$  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  $\text{pH}_{\text{sw}}$  (*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  $^{11}\text{B}$   
348 enrichment levels off for the two lowest  $\text{pH}_{\text{sw}}$  treatments.

349

350  $^{11}\text{B}$  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  $\text{pH}_{\text{sw}}$ , 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  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$ ) observed in cultured *Neogoniolithon* sp., or has a  
361 significant influence on the relationship between skeletal  $\delta^{11}\text{B}$  and  $\text{pH}_{\text{sw}}$  in this species.  
362 Furthermore, although  $^{11}\text{B}$  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  $\text{CaCO}_3$  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  $^{11}\text{B}$ , 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  $\text{CaCO}_3$ . In theory, therefore, Rayleigh fractionation may be sufficient to describe  
385 both the observed enrichment in  $^{11}\text{B}$  in coralline algae calcite relative to seawater borate  
386 (Figure 2), the observed relationships between  $\text{pH}_{\text{sw}}$  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  $\text{CaCO}_3$   $\delta^{11}\text{B}$ .  
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  $^{11}\text{B}$  enrichment in  
404 *Neogoniolithon* sp. relative to seawater borate, as well as the observed relationships between  
405  $\text{pH}_{\text{sw}}$  and  $\delta^{11}\text{B}$ , and B/Ca.

406

#### 407 *4.2 Calcification rate and implications for coralline red algae in a high- $\text{CO}_2$ 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  $\text{pH}_{\text{cf}}$  elevation increasing  $\Omega$  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  $\text{pH}_{\text{cf}}$  pursuant to the  
421  $\delta^{11}\text{B}$ - $\text{pH}_{\text{sw}}$  relationship, as proposed for scleractinian corals (*e.g.* McCulloch et al., 2012b). Since  
422  $\text{pH}_{\text{cf}}$  will largely control calcite saturation state ( $\Omega$ ) at the site of calcification, calcification rate  
423 should exhibit a strong relationship with  $\delta^{11}\text{B}$  and  $\text{pH}_{\text{cf}}$ . 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  $\text{pH}_{\text{cf}}$  (Figure 6A). The observed relationship between coralline algal calcification rate  
426 and  $\text{pH}_{\text{sw}}$  (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  $\text{pH}_{\text{sw}}$  and  $\text{pH}_{\text{cf}}$  (Figure 4). Furthermore, the ability of *Neogoniolithon* algae to raise  $\text{pH}_{\text{cf}}$   
429 relative to  $\text{pH}_{\text{sw}}$  increases under more acidified conditions, with  $\Delta\text{pH}$  increasing from 0.85 ( $\pm$



430 0.11) to 1.26 ( $\pm 0.22$ ) between  $\text{pH}_{\text{sw}}$  of 8.19 to 7.91. These results are consistent with three  
431 coralline algae species (including a crustose *Neogoniolithon* sp.) cultured at variable  $\text{pH}_{\text{sw}}$  by  
432 Cornwall et al. (2017), which also exhibit a similar increase in  $\Delta\text{pH}$  from ca. 0.8 to ca. 1.1  
433 between  $\text{pH}_{\text{sw}}$  of 8.08 to 7.64 (Cornwall et al., 2017). However, our observation that  $\Delta\text{pH}$   
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  $\text{pH}_{\text{cf}}$  relative to  $\text{pH}_{\text{sw}}$ . This limit may  
436 also exist for those species examined by Cornwall et al. (2017) but is not resolvable because  
437 their  $\text{pH}_{\text{cf}}$  data are confined to a narrower  $\text{pH}_{\text{sw}}$  range, with only three  $\text{pH}_{\text{sw}}$  treatments  
438 examined that fall within the linear portion of our  $\text{pH}_{\text{cf}}$  vs.  $\text{pH}_{\text{sw}}$  relationship. Nonetheless, taken  
439 together, our study and that of Cornwall et al. (2017) illustrate that  $\text{pH}_{\text{cf}}$  in coralline algal  
440 therefore appears to promote calcification in moderately acidified seawater (down to  $\text{pH}_{\text{sw}}$   
441 7.95), which is most likely due to  $\text{CO}_2$ -fertilisation of photosynthesis. This supports the previous  
442 observation that photosynthesis in some marine algae is  $\text{CO}_2$ -limited up to ca.  $1000 \mu\text{atm } p\text{CO}_2$   
443 (Bowes, 1993). Our new data at low  $\text{pH}_{\text{sw}}$ , 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  $\Omega$  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  $\text{pH}_{\text{cf}}$  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  $\text{pH}_{\text{sw}}$  optima  
452 exist at  $\text{pH}_{\text{sw}}$  ca. 8 for maximising both  $\text{pH}_{\text{cf}}$  and calcification rates of *Neogoniolithon* sp.  
453 However, that  $\text{pH}_{\text{cf}}$  and algal calcification rates begin to dramatically decline as  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$ ,  
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  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{sw}}$  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  $\text{CaCO}_3$  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  $\text{pH}_{\text{cf}}$  of the algae,  
477 and that there is considerable variability in  $\text{pH}_{\text{cf}}$  amongst individuals.

478

479 The finding that coralline red algal  $\delta^{11}\text{B}$  responds to  $\text{pH}_{\text{sw}}$  suggests that this is a potential taxon  
480 for reconstructing palaeo- $\text{pH}_{\text{sw}}$ ; a conclusion that is particularly noteworthy given coralline  
481 algae's ability to produce long growth records in high-latitude oceans, where palaeo- $\text{pH}_{\text{sw}}$   
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  $\text{pH}_{\text{sw}}$ ,  
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  $\text{pH}_{\text{sw}}$ ,  $\delta^{11}\text{B}$  and skeletal B/Ca, and  $\text{pH}_{\text{cf}}$  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  $\text{pH}_{\text{cf}}$  increase of  
492 more than 1 pH unit relative to  $\text{pH}_{\text{sw}}$ . An observed correlation between calcification rate and  
493  $\text{pH}_{\text{cf}}$  suggests that the algae promote calcification by elevating  $\text{pH}_{\text{cf}}$ . Furthermore, the  
494 observation that  $\Delta\text{pH}$  increased as  $\text{pH}_{\text{sw}}$  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  $\text{pH}_{\text{cf}}$  and calcification rates  
497 decreased when  $\text{pH}_{\text{sw}}$  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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740



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 CO<sub>2</sub>-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  $\Delta pH$  ( $pH_{cf} - pH_{sw}$ ) vs.  $pH_{sw}$  (B).** An apparent parabolic relationship is  
760 observed in A, with a maximum at  $pH_{cf} \sim 7.95$ . In B,  $\Delta pH$  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.

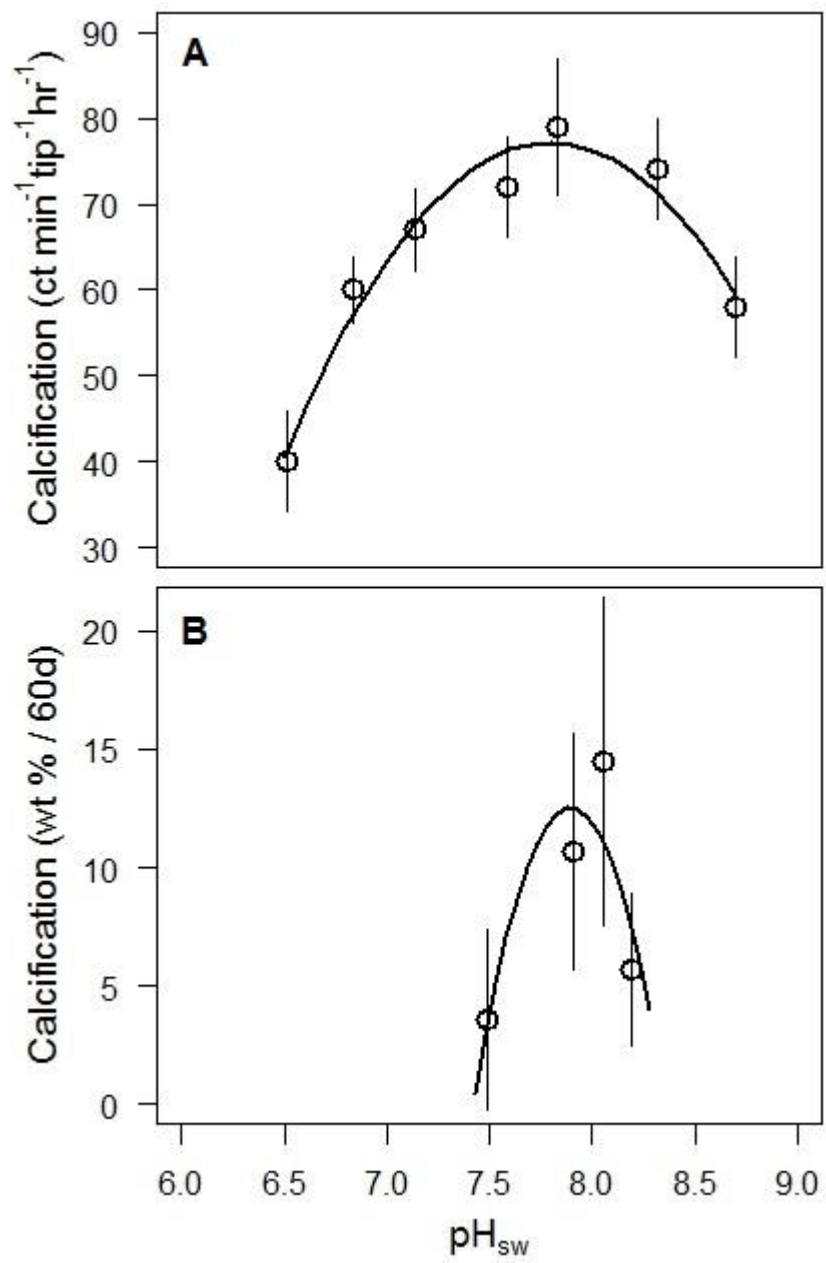
777 *Table captions*

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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,  $1\sigma$  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.

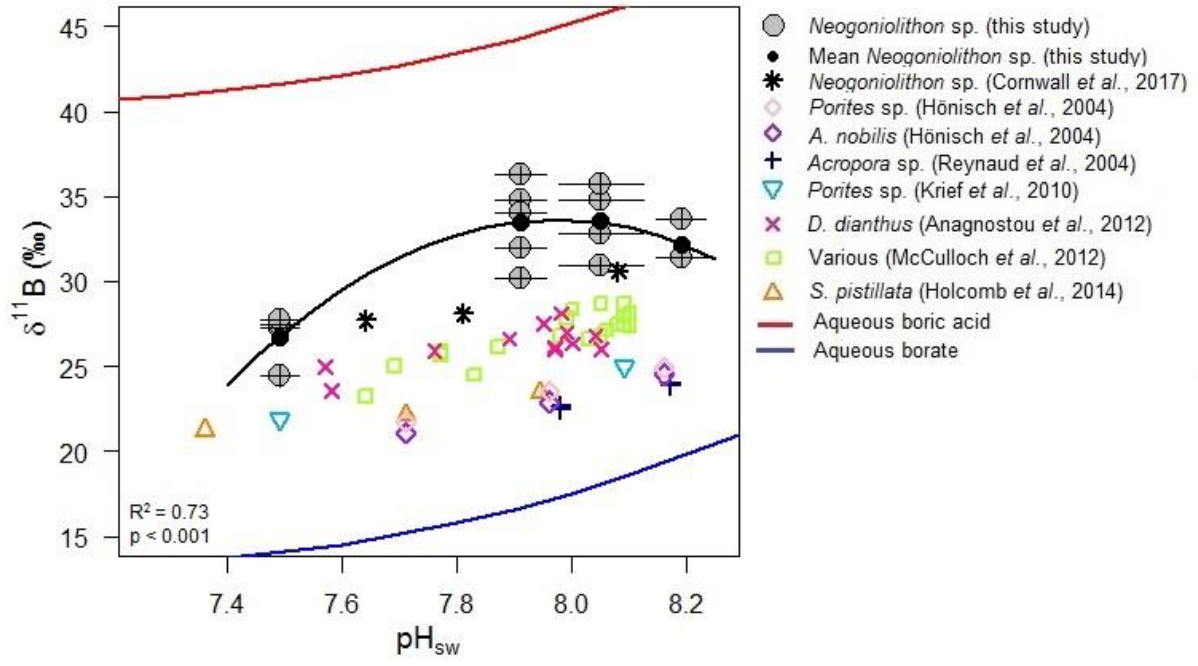
783 **Table 2. Mean skeletal  $\delta^{11}B$  compositions for each experimental treatment, along with percentage**  
784 **of boric acid (enriched in  $^{11}B$  by 27.2 ‰) required to be incorporated into the algal calcite to**  
785 **generate the measured  $\delta^{11}B$  composition, assuming that  $\delta^{11}B$  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  $\Delta pH$  describes the change in pH  
789 according to the equation  $\Delta pH = pH_{cf} - pH_{sw}$ .  $1\sigma$  are shown in parentheses.

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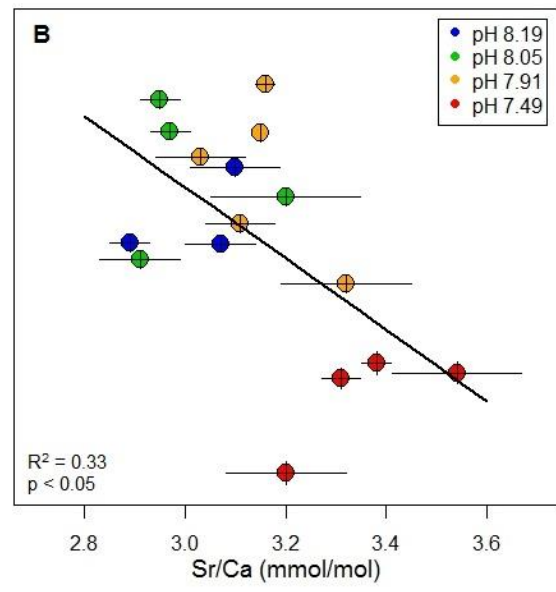
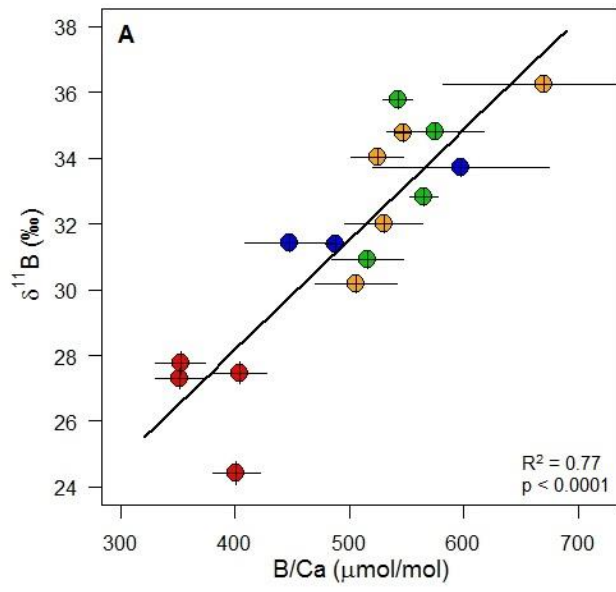
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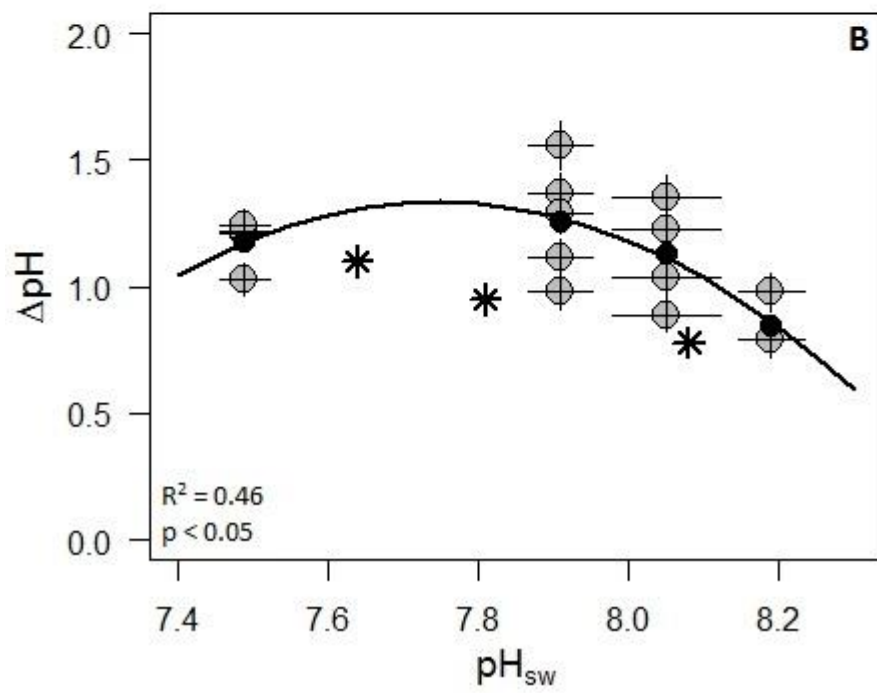
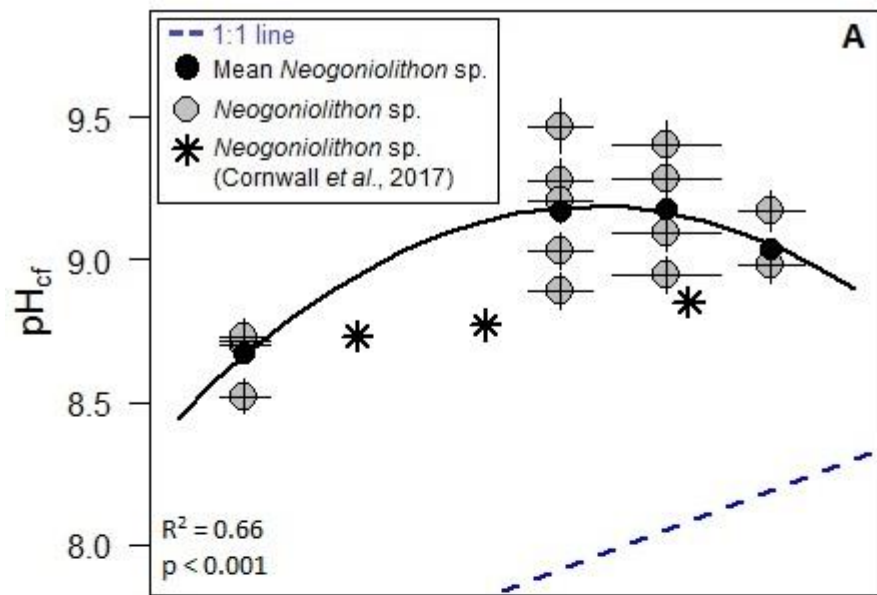
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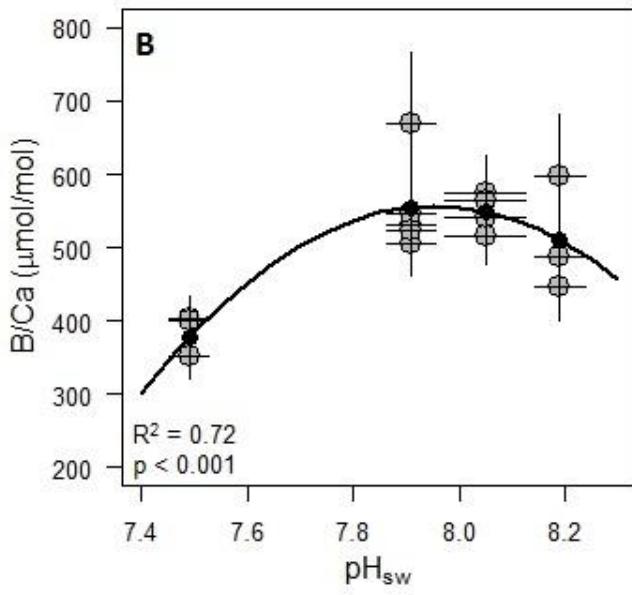
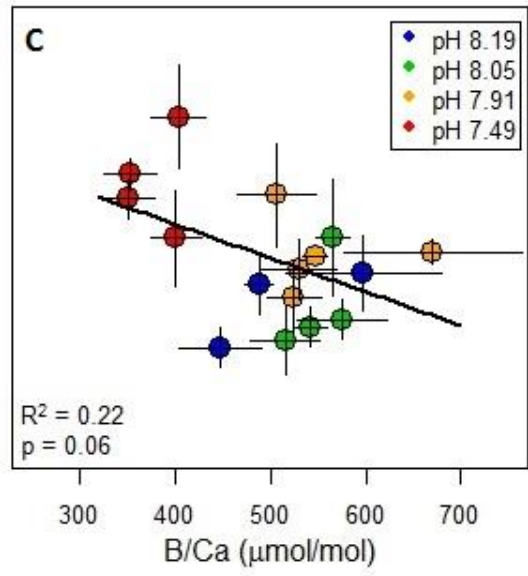
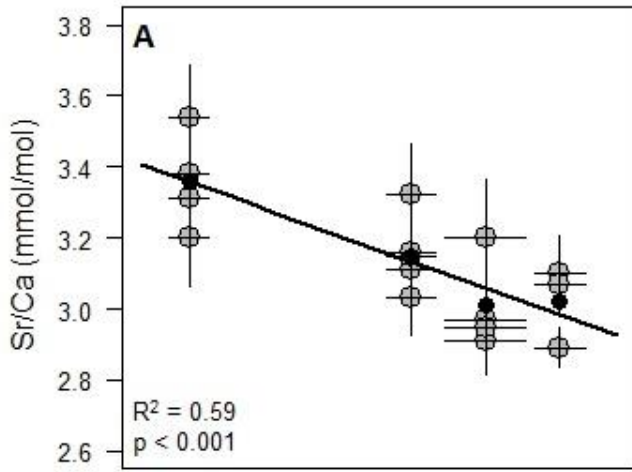
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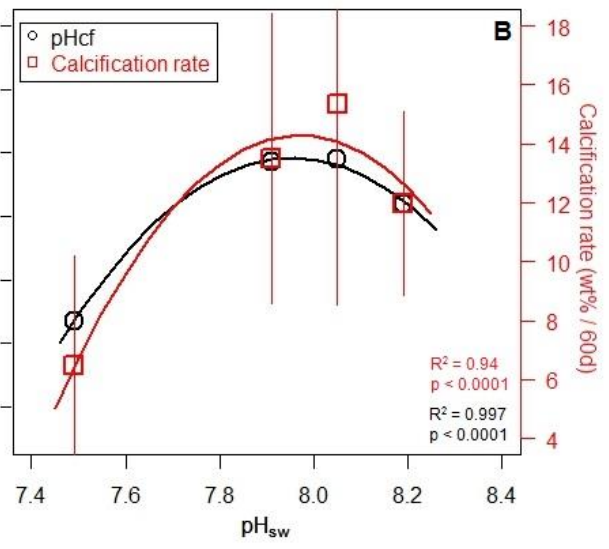
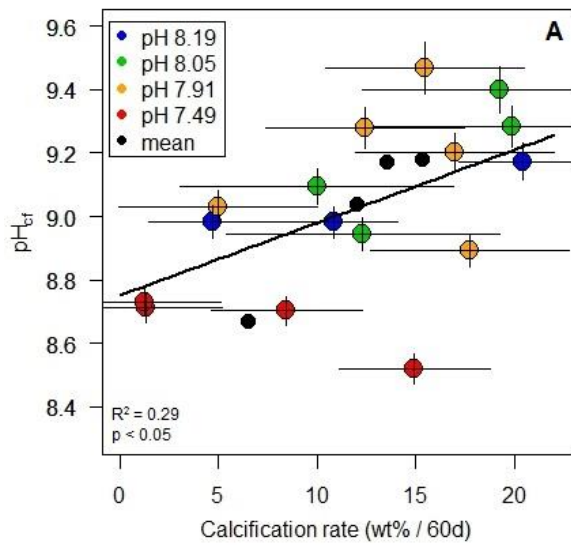
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**Table 1.**

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



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**Table 2.**

<b><math>p\text{CO}_2</math></b> <b><math>\mu\text{atm}</math></b>	<b><math>\text{pH}_{\text{sw}}</math></b>	<b><math>\delta^{11}\text{B}</math></b> <b><math>\text{‰}</math></b>	<b><math>\text{B}(\text{OH})_3</math></b> <b><math>\%</math></b>	<b>Net calcification</b> <b><math>\text{wt}\% / 60 \text{ days}</math></b>	<b><math>\text{pH}_{\text{cr}}</math></b>	<b><math>\Delta\text{pH}</math></b>
<b>409 (6)</b>	8.19 (0.03)	32.17 (1.33)	44 (4)	5.7 (2.9)	9.04 (0.11)	0.85 (0.11)
<b>606 (7)</b>	8.05 (0.06)	33.58 (2.16)	57 (7)	14.5 (6.6)	9.18 (0.20)	1.13 (0.20)
<b>903 (12)</b>	7.91 (0.03)	33.45 (2.38)	60 (8)	10.7 (4.7)	9.17 (0.22)	1.26 (0.22)
<b>2856 (54)</b>	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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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,  $\Omega$  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  $\text{pH}_{\text{sw}}$   
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*

826 Mucci A. (1983) The solubility of calcite and aragonite in seawater at various salinities, temperatures and  
827 one atmosphere total pressure. *Am. J. Sci.* **283**, 780–799.

828 Ries J. B., Cohen A. L. and McCorkle D. C. (2009) Marine calcifiers exhibit mixed responses to CO<sub>2</sub>-induced  
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.

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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 <sup>2</sup> )	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
$\Omega_{arag}$		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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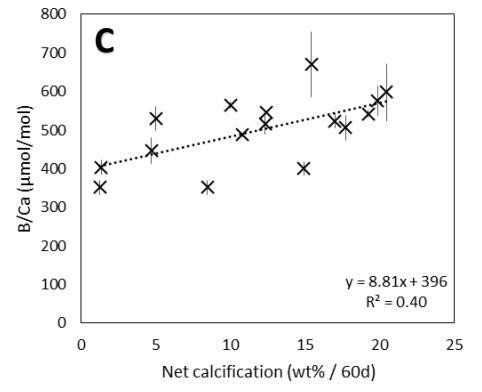
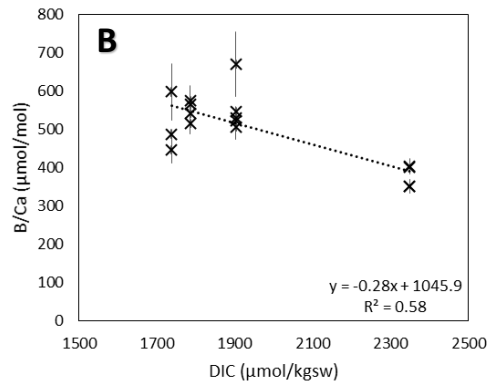
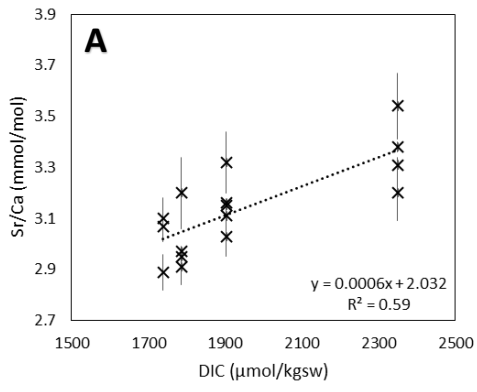
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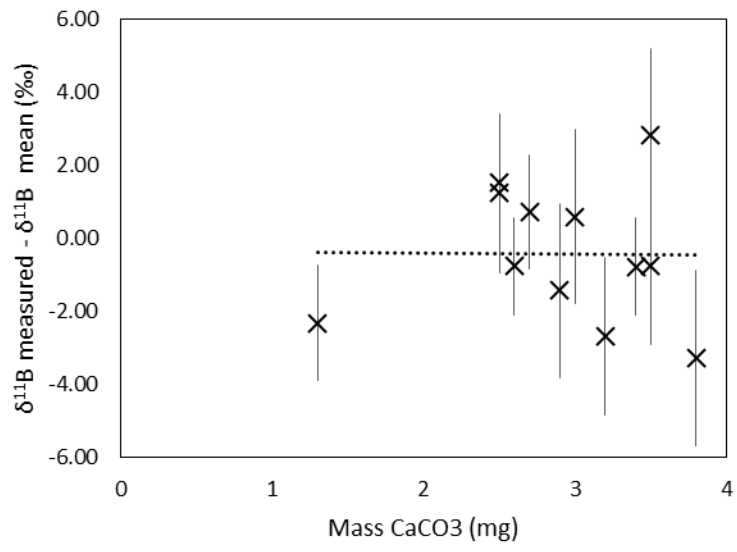
**Table SM2.**

<b>Parameter 1</b>	<b>Parameter 2</b>	<b>Regression</b>	<b>Equation</b>	<b>R<sup>2</sup></b>	<b>p</b>
$\delta^{11}\text{B}$	pH <sub>sw</sub>	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 <sub>cf</sub>	pH <sub>sw</sub>	Curve	$y = -2.4x^2 + 38.5x - 143.8$	0.66	< 0.001
$\Delta\text{pH}$	pH <sub>sw</sub>	Curve	$y = -2.4x^2 + 37.6x - 144.3$	0.46	< 0.05
Sr/Ca	pH <sub>sw</sub>	Linear	$y = -0.53x + 7.3$	0.59	< 0.001
B/Ca	pH <sub>sw</sub>	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 <sub>cf</sub>	Calcification rate	Linear	$y = 0.023x + 8.8$	0.29	< 0.05
<b>Calcification rate</b>	pH <sub>sw</sub>	Curve	$y = -34x^2 + 545x - 2161$	0.94	< 0.0001

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