Mineralogical response of the Mediterranean crustose coralline alga *Lithophyllum cabiochae* to near-future ocean acidification and warming

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Abstract

Red calcareous coralline algae are thought to be among the organisms most vulnerable to ocean acidification due to the high solubility of their magnesium calcite skeleton. Although, skeletal mineralogy is proposed to change as CO_2 and temperature continues rising, there is currently very little information available on the response of coralline algal carbonate mineralogy to near-future changes in pCO_2 and temperature. Here we present results from a one-year controlled laboratory experiment to test mineralogical responses to pCO_2 and temperature in the Mediterranean crustose coralline alga (CCA) *Lithophyllum cabiochae*. Our results show that Mg incorporation is mainly constrained by temperature (+1 mol% $MgCO_3$ for an increase of 3°C) and there was no response to pCO_2. This suggests that *L. cabiochae* thalli have the ability to buffer their calcifying medium against ocean acidification, thereby enabling them to continue to deposit Mg-calcite with a significant mol% $MgCO_3$ under elevated pCO_2. Analyses of CCA dissolution chips showed a decrease in Mg content after 1 year for all treatments but this was not affected by pCO_2 nor by temperature. Our findings suggest that biological processes exert a strong control on calcification on Mg-calcite and that CCA may be more resilient under rising CO_2 than previously thought. However, previously demonstrated increased skeletal dissolution with ocean acidification will still have major consequences for the stability and maintenance of Mediterranean coralligenous habitats.

Keywords: Ocean acidification, carbonate skeleton, coralline algae, global warming, mineralization, Mg-calcite, CO_2, temperature

1. INTRODUCTION

Coralline algae are thought to be among the organisms most vulnerable to ocean acidification (decreasing pH and increasing pCO_2). This is because their skeletons consist of magnesium-calcite (Mg-calcite) and the solubility of Mg-calcite (> 8-12 mol% $MgCO_3$) is greater than the solubility of the other forms of calcium carbonate ($CaCO_3$), calcite (low Mg-calcite, <8 mol%) and aragonite (Andersson et al., 2008). Consequently, it has been suggested that coralline algae, both articulated and crustose coralline algae (CCA), will be among the first organisms to dissolve in the context of ocean acidification (Andersson et al., 2008). However, the presence of Mg-calcite phases with lower solubility such as dolomite (50 mol% $MgCO_3$) within the cells of tropical CCA, results in reduced dissolution rates (Kline et al., 2012; Nash et al., 2013a). Potential resilience of coralline algae to ocean acidification may thus occur through changes in skeletal mineralogy either by producing calcite with lower Mg content (Chave, 1954; Agegian, 1985; Stanley et al., 2002; Ries, 2011; Egilsdottir et al., 2013) or by favoring accumulation of $CaCO_3$ forms with lower solubility such as dolomite (Diaz-Pulido et al., 2014). The Mg content in coralline algae is also known to vary as a function of seawater temperature (Agegian, 1985; Halfar et al., 2000; Kamenos et al., 2008; Hetzinger et al., 2009; Caragnano et al., 2014; Diaz-Pulido et al., 2014), which is considered to exert a primary control by facilitating Mg incorporation into the skeleton (Kamenos et al., 2008). However, there is currently limited information available on the response of the mineralogy of coralline algae to near-future changes of pCO_2 and temperature, and none on temperate CCA.

The response of dead CCA crust to varying pH conditions is also of interest as it is the preservation of this crust that underpins many of the coralligenous habitats. It has been proposed that as CO_2 rises, calcite with higher Mg-contents will dissolve and may re-precipitate as lower Mg-phases (Andersson et al., 2008). This would result in lower average mol% $MgCO_3$ of Mg-calcite. As it is proposed than calcite with lower Mg-contents are more thermodynamically stable that those with higher Mg-content, that could provide a positive feedback mechanism to stabilize the calcium carbonate skeletons. As yet, there has been no experimental work on the Mg-calcite skeletons of CCA to test this proposal. An experiment performed on skeletal chips of *Lithophyllum cabiochae* reported rates of dissolution 2 to 4 times higher under elevated pCO_2 than under ambient pCO_2 (Martin and Gattuso 2009). These dissolution chip samples offer an opportunity to test the theory that higher Mg phases of Mg-calcite would dissolve differentially from the CCA crusts potentially increasing the stability of the dead substrate.

We investigated experimentally the response of the carbonate mineralogy of the CCA *Lithophyllum cabiochae*, one of the main calcareous components of coralligenous habitats in the Mediterranean Sea, after 12 months of exposure to ocean acidification and warming. The hypotheses tested are: (1) the Mg content of the new growth would increase with temperature, (2) the Mg content of the new growth would decrease under elevated pCO_2, and (3) the Mg content of dead dissolution chips would decrease with elevated pCO_2.

2. MATERIALS AND METHODS

Full experimental details, carbonate chemistry, growth, respiration, photosynthesis, net calcification and dissolution rates can be found in Martin and Gattuso (2009) and Martin et al. (2013a). A summary follows. Specimens of the CCA *Lithophyllum cabiochae* (Boudouresque & Verlaque) Athanasiadis were collected in the coralligenous community at *ca.* 25 m depth in the Bay of Villefranche (NW Mediterranean Sea, France; 43°40.73'N, 07°19.39'E) on 10 July 2006 and transported to the laboratory in thermostated tanks within 1 h. Flat thalli were selected for the experiments and were thoroughly cleaned of epiphytic organisms. They were randomly assigned in four 26-L aquaria and reared for one year (July 2006-August 2007) in four treatments:

(1) ambient pCO_2 (*ca.* 400 µatm) and ambient temperature (*T*, *i.e.* the temperature at 25 m depth in the Bay of Villefranche; control, labeled *400 T*),

(2) ambient pCO_2 and elevated temperature (*T+3*°C; *400 T+3*),

(3) elevated pCO_2 (*ca.* 700 µatm) and ambient temperature (*700 T*),

(4) elevated pCO_2 and elevated temperature (*700 T+3*).

A further set of CCA thalli were air dried until dead and placed in the tanks in December 2006 for the remaining 8 months of the experimental period to measure rates of dissolution (Martin and Gattuso, 2009). The aquaria were continuously supplied with Mediterranean seawater from two 110-L header tanks in which pCO_2 was adjusted by bubbling ambient air (ambient pCO_2) or CO_2-enriched air (elevated pCO_2) obtained by mixing pure CO_2 to ambient air. Temperature was gradually changed according to the season from *T* = 13.3 to 22.0°C (*T+3* = 16.3 to 25.0°C). Irradiance was set to the mean *in situ* daily irradiance at 25 m depth in the Bay of Villefranche and was adjusted seasonally from 6 to 35 µmol photons m^{-2} s^{-1}. The photoperiod was adjusted weekly according to natural fluctuations and varied from 9:15 (Light:Dark ratio - winter) to 15:9 (summer). The annual means of the carbonate chemistry parameters are shown in Table 1.At the end of the experiment all crusts

were air-dried. Four sets of crust were sampled for X-ray diffraction (XRD): (1) the new crusts grown from the bottom face of the main thalli (Figure 1), (2) the pink surficial crust on the original thalli, (3) the original thalli (Fig. 1) and (4) pieces of dead crust that had been used for dissolution tests. New crusts were confirmed to have grown during the experiment as the pre-existing crust was cleaned and photographed at the time of collection and these growths were not present at that time. For the new crusts, sets of 4-5 crust fragments similar in size (*ca*. 2-3 mm in diameter) and thickness (~ 1 mm thick), were randomly selected from 8 thalli per treatment. To obtain sufficient material for XRD analyses of the new thalli, 3-4 crust fragments were used from each alga. For the original thalli and dissolution chips, subsamples ~2-3 mm thick were cut off the sides. The pink surface of the original thalli was sampled by gently scraping with a razor ensuring not to scrape into the white crust underneath. This uppermost surface was presumed to have grown during the experiment and sampled instead of the surfaces of the new crusts as there was not a large enough surface area on the new protrusions to collect sufficient pink crust for analyses. Scrapings from 5 algae from each treatment were required in order to obtain enough material for one XRD test. The depth of the pink-pigmented crust was ~200- 500 µm but only the surface is sampled by scraping on the top with the aim of collecting predominantly epithallial material. However, we do not refer to it as epithallus because by this sampling method we cannot confirm that no sub-perithallial crust has been included, hence surficial pink crust is the most accurate description of this subsample. Our development of this method has shown that if too much pressure is applied during the scraping then substantial amounts of perithallial crust, that also can be pink-pigmented, may be unintentionally sampled.

The mol% $MgCO_3$ of the crust fragments were determined via XRD using a Siemens D501 Bragg-Brentano diffractometer equipped with a graphite monochromator and scintillation detector, using Cu$K\alpha$ radiation. Crust fragments were crushed and powdered with fluorite (CaF_2) added as an internal standard. The coralline crust and fluorite are ground together with a mortar and pestle. Enough fluorite was added to obtain a clear peak, this is usually between 5-20 weight percent but not specifically weighed. Powdered samples were mounted on a low background quartz slide. For the pink surficial crust, more fluorite (30-50 %) was added to obtain enough powder to cover the centre of the quartz slide. Mg-content of calcite was calculated from the (104) peak position ~~as described in Nash et al. (2013b)~~and any asymmetry present was quantified as described in Nash et al. (2013b). XRD scans with 25-32° 2-theta scan length were processed using EVA Diffract Plus software packages and interpreted following procedures described Nash et al. (2013b) and further developed in Diaz-Pulido et al. (2014). XRD measurements had a reproducibility of ± 0.11 mol% (standard deviation; n=3 analytical repeats of sample 700T+3, 5a).

The effects of pCO_2 and temperature were assessed by two-way ANOVAs and followed by Tukey HSD post hoc tests. Normality of the data and homoscedasticity were checked by Kolmogorov-Smirnov's test and Levene's test, respectively. A t-test was completed to compare asymmetry differences between the main thalli and dissolution chips.

3. RESULTS

In general, the Mg content of the CCA's increased with temperature but was not affected by CO_2 (Fig. 2). Dissolution chips had lower Mg content than the main thalli and neither the main thalli (pre-experimental crust) or the dissolution chips showed any trends with temperature or CO_2 (Table 2).

3.1 New crust- XRD results indicate that the new crusts of *L. cabiochae* are entirely calcitic (Mg-calcite). The mean (±standard deviation) Mg contents were 15.2 ± 0.7, 16.0 ± 0.5, 15.0 ± 0.5, and 16.1 ± 0.3 mol% $MgCO_3$ in the *400 T*, *400 T+3*, *700 T*, and *700 T+3* treatments, respectively (Fig. 2, the complete data set is provided in Supplementary information table 1). The Mg-calcite peaks were symmetrical indicating there was no dolomite, nor magnesite present. The Mg content was significantly affected by temperature (2-way ANOVA, $p < 0.0001$), being about 1 mol% $MgCO_3$ higher at elevated temperature (+3°C) relative to ambient temperature at both pCO_2 levels but was not affected by pCO_2 (Fig. 2; Table 2A).

3.2 Pink surficial crusts- The pink surficial crusts were also entirely Mg-calcite. The Mg content was 14.3, 14.6, 14.6 and 15 mol% $MgCO_3$ in the *400 T*, *400 T+3*, *700 T*, and *700 T+3* treatments, respectively (Fig. 2). There is no standard deviation or statistical analysis of the pink surficial crust results because only one analysis was performed on ~~the combination of~~ material from 5 thalli combined for each treatment.

3.3 Main thalli- The mean (± standard deviation) Mg content in the main thalli were 16.0 ± 0.5, 16.1 ± 0.4, 15.6 ± 0.4, and 16.1 ± 0.6 mol% $MgCO_3$ in the *400 T*, *400 T+3*, *700 T*, and *700 T+3* treatments, respectively. The Mg content was not affected by temperature or pCO_2 (Table 2B, SI. Table 2). There was minor asymmetry on the higher mol% $MgCO_3$ side of Mg-calcite XRD peaks indicating the presence of a higher Mg-calcite phase (Fig. 3). However, this asymmetry did not extend over the dolomite position suggesting the extra phase was a second Mg-calcite. The difference in mol% $MgCO_3$ when incorporating the extra asymmetry into the calculations (see Nash et al. 2013b for full discussion on this method) showed that the asymmetry was also not affected by temperature or pCO_2 (Table 3).

3.4 Dissolution chips- The mean (± standard deviation) Mg content of dissolution chips were 15.4 ± 0.5, 15.6 ± 0.5, 15.6 ± 0.5, and 15.5 ± 0.4 mol% $MgCO_3$ in the *400 T*, *400 T+3*, *700 T*, and *700 T+3* treatments, respectively. The Mg content was not affected by temperature or pCO_2 (Table 2C, SI Table 3). The average Mg content was significantly lower in the dissolution chips than in main thalli (15.5 ± 0.4 versus 16.0 ± 0.5 mol% $MgCO_3$, t-test, $p < 0.001$) (Fig. 3 A, B). Similarly to the main thalli, there was a minor asymmetry on the higher mol% $MgCO_3$ side of the Mg-calcite XRD peak indicating a second phase of Mg-calcite with higher Mg content (Fig. 3 B). The difference in asymmetry was lower for the dissolution chips than the main thalli (t-test, $p = 0.008$; Fig. 3 C) and was not affected by temperature or pCO_2 (Table 3).

4. DISCUSSION

Results obtained on the new crust demonstrate that the mineralogy of *L. cabiochae* is primarily controlled by temperature and scarcely constrained by pCO_2. Similarly, the Mg content does not respond to pCO_2 in dead CCA skeleton but decreases in all dead crusts over the 12-month experiment. Thus our hypothesis that the Mg content would increase with temperature is supported but the hypothesis that Mg content would decrease with pCO_2 is not. Seawater temperature is effectively considered to exert primary control on Mg content in coralline algae (Halfar et al., 2000; Kamenos et al., 2008). In *L. cabiochae*, an increase of 3°C above ambient temperature led to an increase in Mg incorporation of 1 mol% $MgCO_3$, (0.33 mol% $MgCO_3$/°C) which is consistent with the values reported in the literature, both experimentally and *in situ*, ranging between 0.3 and 2 mol% $MgCO_3$ per °C (Chave and Wheeler, 1965; Halfar et al., 2000; Kamenos et al., 2008; Hetzinger et al., 2009; Caragnano et al., 2014; Diaz-Pulido et al., 2014; Williamson et al., 2014). Conversely, pCO_2 did not drive significant ~~mineralogical~~ change in the Mg content of living *L. cabiochae*.

The Mg content of the pink surficial crust was higher in the elevated temperature treatments and while no statistical analyses could be carried out, these results are consistent with the increase in Mg measured for pink surficial crust as a function of increasing temperature reported in previous work (Diaz-Pulido et al., 2014). The lower Mg content recorded for the pink surficial crust relative to the bulk crust is in agreement with previous studies on CCA *Porolithon onkodes* (Diaz-Pulido et al., 2014; Nash et al., 2015). Sampling of the surface aims to capture predominantly epithallial carbonate. The epithallial cells of corallines are typically different in shape to the perithallial cells, being shorter and flattened or ovoid shape (e.g. Pueschel and Keats 1997). It is not presently known why the epithallus has a lower Mg than the bulk perithallus or if this offset is common to all

species. However, the close agreement of the temperature response for the *P. onkodes* surficial crust of 0.37 mol% $MgCO_3$/°C (Diaz-Pulido et al. 2014) with the 0.33 mol% $MgCO_3$/°C measured for the new crust in this experiment does suggest the controls on Mg uptake are similar for both the perithallial and epithallial cells.

The consistent shift of ~0.33 mol% $MgCO_3$/°C across both the control and CO_2 treatments, consider together with the results for Diaz-Pulido et al. (2014) of 0.37 mol% $MgCO_3$/°C from a 2°C increase -suggests the magnesium change is a robust temperature response and this is of interest for CCA paleo temperature proxies (e.g. Halfar et al. 2000, Kamenos et al. 2008, Hetzinger et al., 2009). This increase in Mg content is also in agreement with results obtained by XRD of articulate corallines [0.286 – 0.479 mol% $MgCO_3$/°C (Williamson et al. 2014)] and a variety of species [0.36 mol% $MgCO_3$/°C using only XRD results in Chave (1954)] collected across a geographical temperature range. The reports of ratios of up to 2 mol% $MgCO_3$/°C (Halfar et al., 2000: Kamenos et al., 2008, Hetzinger et al., 2009; Caragnano et al., 2014; Williams et al. 2014) may be due to different analytical methods or species-specific effects. The similarity of the results obtained using XRD for both experimental and *in situ* corallines supports using a ratio of ~0.3 to 0.4 mol% $MgCO_3$/°C as a paleo thermometer when the analytical methods return an effective spatial average for Mg-calcite and the absence of other carbonates; aragonite, low-Mg-calcite, dolomite and magnesite, has been confirmed.

The new crusts represent the average mol% $MgCO_3$ influenced by the temperature experienced during their growth, which is unlikely to be an even representation of the entire experimental duration. This is for two reasons. First, growth rate varies with temperature which could result in a bias towards warmer months Mg-content. Secondly, although the progression of the new crust growth was not specifically monitored throughout the experiment, it is likely that a relatively small amount of the final material would have formed during these first few months. For the new growths to form, initially new hypothallial cells would have to bud out and then change to perithallial cells, which form the bulk of CCA crust material. The experiment started and finished in summer, July. The warmest months were July, August and September (Martin et al. 2009). Calcification rates during autumn, winter, spring ranged from ~0.02 – 0.15 μmol $CaCO_3$ $cm^{-2}h^{-1}$, in contrast to the substantially higher rates of 0.3 – 0.52 μmol $CaCO_3$ $cm^{-2}h^{-1}$ during summer months (Martin et al. 2013). While growth may have commenced during the summer, probably most of the crust formed over the remainder of the experimental period, autumn, winter and spring. However, that there was no significant difference in mineralogy between the

treatments except for the temperature influence, suggests that all crusts analysed grew over similar time frames, i.e. each crust has similar proportions of growth from the differing temperature and time periods.

Prior to utilizing CCA as a temperature proxy, it is necessary to verify that the Mg in the Mg calcite is the primary Mg incorporated during calcification and not the result of diagenesis. The depletion of Mg in the dissolution experiment crusts over eight months indicates this change can be relatively rapid once the organism is no longer protected by living tissue. This is likely to be more of a problem when using fossil branching corallines than thick crusts that retain a living surface layer. Indeed, Kamenos et al. (2008) noted their sub-fossil *Lithothamnion glaciale* had significantly lower Mg in the summer season than their living samples. The likelihood of remineralization was considered by Kamenos et al. but rejected, as remineralization was presumed to be to either low Mg calcite or aragonite. The possibility of remineralization to a lower phase of Mg-calcite, as occurred in this experiment and noted at the base of CCA *Porolithon onkodes* (Nash et al. 2013b) had not previously been reported. The present study experimentally confirms that diagenesis does not necessarily mean a change to aragonite or low Mg-calcite, but instead can be to a lower phase of Mg-calcite thus making it more difficult to detect post-mortem diagenesis from Mg measurements or mineralogy alone. High magnification SEM work would be required to check for remineralization.

Analyses of the pre-existing thalli (main thalli) provide a baseline Mg content for pre-experimental *L. cabiochae* with the assumption that this has not changed during the experiment. The average across treatments was 16.1 mol% $MgCO_3$, excluding the 700T treatment. This Mg content is higher than that of the new crusts grown under ambient temperatures (400T and 700T). ~~This~~ and is probably due to a larger amount of pink surficial crust with lower Mg content in the thin new crusts relative to the pre-existing thicker thalli. Although the lower average for 700T is not significantly different from the other three treatments, when this lower Mg content is considered in the context of the results for the dissolution chips the lower measurement takes on greater relevance. Results for the dissolution chips were not significantly different between treatments with a combined average of 15.5 mol% $MgCO_3$. This was significantly lower than the pre-existing thalli for all treatments except the 700T, suggesting the 700T main thalli may have undergone alteration similarly to the dissolution chips during the experiment. The values for 700T were compared by t test to the combined dataset for 400T, 400T+3 and 700T+3 and were significantly lower than the group, $p = 0.0531$.

Ideally when carrying out experiments where it is planned to analyse the crust, for either mineral or structural changes, it is best if a subsample is taken from of each piece prior to being placed into the experimental tanks. This way it can be established that post-experiment crust features are truly representative of the environmental sample (e.g. Nash et al. 2013a) and have not been altered by virtue of being placed in tanks for the duration as is suspected for 700T. Problematically some CCA exhibit changes in growth unrelated to treatment after being placed in tanks (Nash et al. 2015). Thus best practice would be to keep aside subsamples, particularly where a species has not already been well studied at the cellular scale, so that it can be determined if the control tanks result in growth and mineral composition comparable to in-situ growth.

It is interesting to consider why the dissolution chips have lower Mg content than the main thalli when they were subsamples of the same. Presumably, because the thalli remained covered in living tissue, this has substantially protected the crust from exposure to ambient seawater whereas the dissolution chips had direct exposure to seawater. Assuming that the dissolution chips initially had the same Mg content as the main thalli from which they were subsampled, then, the lower Mg content after 8 months of direct exposure to seawater indicates there has been alteration of the crust. All chips lost weight over the 8 months (Martin and Gattuso 2009) with those in the 700T and 700T+3 treatments having the highest dissolution rates. However, the absence of a trend for Mg content with treatment indicates that dissolution rates do not influence the thermodynamics of the Mg-calcite dissolution process for these CCA. The strong correlation with CO_2 and dissolution rates in the original experiment (Martin et al. 2009) suggests that the seawater pH is the dominant factor in driving dissolution. Indeed micro-bioerosion can increase in response to that, as shown in Diaz-Pulido et al. (2014), but the mechanism by which they could influence the Mg-content of the Mg-calcite is not yet known. Further work is planned to analyse these dissolution chips by SEM-EDS which may shed light on the process.

Theory suggests that the higher phases of Mg-calcite will dissolve first (Andersson et al., 2008) but micro-structural properties may interfere with a purely thermodynamic response (Morse et al., 2007; Henrich and Wefer, 1986; Walter and Morse, 1985, reviewed in Eyre et al., 2014; Pickett and Andersson, 2015). The lowest phase in the *L. cabiochae* is the pink surficial crusts but they do not make up a substantial amount of the main thalli bulk sample. The presence of asymmetry indicates an extra phase of Mg-calcite with a higher content of Mg. Previous works on cold water (Adey et al., 2014) and tropical (Nash et al., 2013a) CCA have shown that the cell wall and inter-filament regions have visually different crystal morphology. It may be that they have different

281 Mg content although this hypothesis has not been tested yet. Statistical results showed lower asymmetry for the
282 dissolution chips compared to the main thallus. This indicates that the relative proportion of higher-Mg-phase
283 Mg-calcite was less in the dissolution chips suggesting that the higher-Mg-phase, while still present, had
284 suffered greater dissolution relative to the lower-Mg-phases. Dissolution experiments have demonstrated that the
285 inter-filament Mg-calcite is the first to dissolve in pH 8 (NBS) after 1 h (Nash et al., 2013) and the cell walls
286 remain intact until exposed to pH 7.7-7.82 over several hours. The pH in the present experiment did not drop
287 below pH_T 7.8 in the 700T or 700T+3 treatments (Martin and Gattuso, 2009). Considering these previous
288 studies and the data presented here, it seems likely that the cell walls have remained substantially intact but the
289 inter-filament Mg-calcite has remineralized to a lower phase of Mg-calcite and there may also be abiotic Mg-
290 calcite infilling cell spaces prior to complete dissolution of the exposed edge. The process of cell infill by Mg-
291 calcite has been observed in the exposed bases of tropical CCA *P. onkodes* (Nash et al., 2013a) whereby exposed
292 dead cells are in-filled with Mg-C. XRD analyses of the exposed base of the tropical CCA measured 14.8 mol%
293 $MgCO_3$ compared to the main crust of 16.9 mol% $MgCO_3$ (Nash et al., 2013b) indicating that the abiotic Mg-
294 calcite has lower average Mg content than the original crust.
295
296 If the proposal for remineralization of the dissolution chips is correct, then the results for the present study would
297 indicate that there is no trend with Mg and temperature or CO_2 for abiotic mineral formation. This would be in
298 contrast to results for synthetic formation of Mg-calcite (Mucci 1987) although the trend for synthetic Mg
299 content was substantially less sensitive than uptake for biogenic Mg-calcite, with an increase of only 2 mol%
300 $MgCO_3$ from 6 to 8 mol% $MgCO_3$ from 5 to 25°C. Support is provided for the absence of temperature trend by
301 another comparison of the results for the dissolution chips to dead tropical CCA sampled from a coral reef core
302 from Rodrigues Island, Indian Ocean (Rees et al., 2005) where the Mg content of the dead crusts was 15 to 15.3
303 mol% $MgCO_3$ (Nash et al., 2013b). To thoroughly test the hypothesis for an absence of temperature trend in
304 abiotic Mg-calcite mineralization, a comprehensive survey of dead CCA from a range of latitudes would be
305 required. However, the clear trend for increase in Mg uptake by living CCA as temperature increases, compared
306 to the absence of trend in altered dissolution chips, suggests the Mg content increase may be primarily driven by
307 a biological response, rather than abiotic thermodynamics alone that the organism is unable to compensate for as
308 suggested by Diaz-Pulido et al. (2014).
309

Although earlier studies on Mg incorporation in the skeleton of coralline algae grown experimentally have found a decline in Mg content with higher pCO_2, likely conferring them a better resistance to dissolution (Agegian, 1985; Ries, 2011; Egilsdottir et al., 2013; Ragazzola et al., 2016), the lack of a pCO_2 effect in *L. cabiochae* is consistent with recent findings (Kamenos et al., 2013; Diaz-Pulido et al., 2014; Nash et al., 2015) suggesting that skeletal mineralogy may be under biological control. The organic substrate may template a baseline magnesium proportion, which then only changes in response to temperature. The ability of coralline algae to control the carbonate chemistry (pH/pCO_2 and carbonate saturation state) of the calcifying medium through metabolic activities could enable them to continue to deposit Mg-calcite with a relatively high mol% $MgCO_3$ despite changes in the carbonate chemistry driven by ocean acidification (Kamenos et al., 2013, Diaz-Pulido et al., 2014) as has been inferred for other Mg-calcite organisms (Ries 2011). A biological control of mineralization by coralline algae has already been inferred in *L. cabiochae* because its rate of calcification is maintained or even enhanced under elevated pCO_2 (Martin et al., 2013a).

It is worth considering whether there is a compensatory mechanism enabling Mg-content maintenance in the elevated pCO_2 treatment. This consideration implies that the Mg content automatically declines with lower pH (the hypothesis we tested) and the organism must therefore have compensated because the results showed no difference with pCO_2. While there have been many studies on Mg content responses to elevated pCO_2 treatments (e.g. Ries, 2011; Egilsdottir et al., 2013; Kamenos et al., 2013; Diaz-Pulido et al., 2014; Nash et al., 2015; Ragazzola et al., 2016) as yet, there has been no study on the internal cellular-scale metabolic controls on Mg uptake in coralline algae. That is, the controls of Mg uptake are unknown. Is it the internal carbonate chemistry, the type of organic substrate or a combination of both? Without an understanding of the physiological mechanisms that control Mg uptake, it is impossible to do more than speculate about potential compensatory metabolic processes. This inhibition to carrying out an informed analysis of potential metabolic controls on Mg uptake highlights the need for basic scientific investigation into how coralline algae calcify and what role the anatomy and organic substrates play in calcification and Mg uptake. Then, we could start to understand how these processes react to external environmental changes. Ragazzola et al. (2013, 2016) and Hoffman et al. (2012) have shown anatomical changes in response to pCO_2 that may be ameliorated over longer time periods but the exact controls on those changes are not known. Recent work has demonstrated the capacity of CCA to maintain elevated pH in the boundary layer (~ 100 microns thick, dependent on water motion) when ambient seawater pH is reduced (Hofmann et al. 2016). The maintenance of the Mg content, if it is related to pH or saturation state,

may be enabled by the organisms capacity to control boundary layer pH and thus effectively inhibit the treatment pH from reaching the living surface.

It remains unclear to what extent the algal metabolism exerts a control on Mg-carbonate chemistry as different effects of pCO_2 on the Mg content and calcification rates have been found in other species of coralline algae (Ries, 2011; Ragazzola et al. 2013, 2016). The increase in Mg content at elevated temperature may lead to increased thalli dissolution but this could be offset by increased calcification (Martin et al., 2013a). However, the enhanced mortality under the combination of projected ocean warming and acidification (Martin and Gattuso, 2009) could have major consequences for the physical stability and maintenance of coralligenous habitats that outweigh any adaptive mineral response. Further work to understand the process that leads to lower Mg content in the dead algal chips post mortem would shed light on remineralization of CCA post-mortem.

Author contributions

S.M. and J.P.G conceived and carried out the experimental work. M.N. carried out the mineral analyses. All authors contributed to writing the MS.

Data availability

All raw data used for statistical analyses is included in the supplementary information.

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Figures

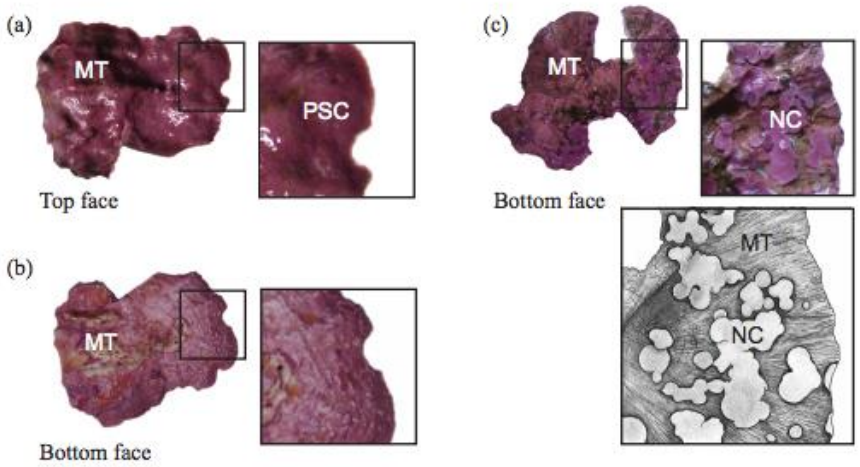

Figure 1: (a) Top face of the main thallus (MT) of *L. cabiochae* showing the pink surficial crust (PSC) and bottom faces (b) free of crusts at the time of collection and (c) with new crusts grown during the experimental period (photos and drawing S. Martin)

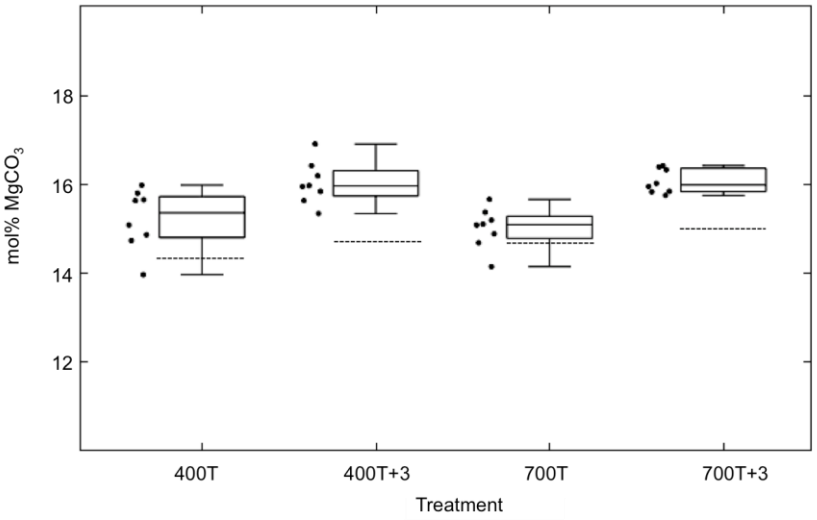

Figure 2: XRD results for new crust and pink surficial growth from the 400 and 700 μatm treatments, in ambient temperature and ambient + 3 °C. Dashed lines shown the mean mol% $MgCO_3$ for pink surficial growth. The box plots represent the new crust and the dots are individual data points. The boxes represent 25 and 75 percentiles, the horizontal bold line is the median value and the whiskers are minimum and maximum values.

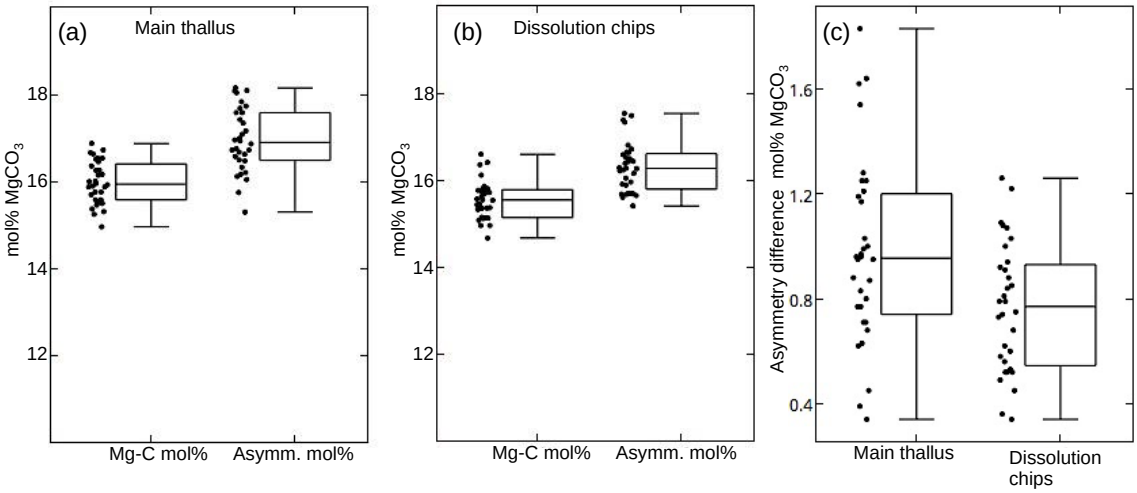

Figure 1: XRD results for main thalli and dissolution chips. **(a)**. Mol% $MgCO_3$ and asymmetry mol% $MgCO_3$ for the main thallus. **(b)**. Mol% $MgCO_3$ and asymmetry mol% $MgCO_3$ for the dissolution chips. **(c)**. Difference in asymmetry mol% $MgCO_3$ between the main thallus and dissolution chips indicating a reduction in the higher phases of Mg-calcite after dissolution.

476 **Table 1** Parameters of the carbonate system in each treatment.

477 The values reported are means (± standard error) of 191 to 194 data collected from July 2006 to August

478 2007. The pH (pH_T, on the total scale) and total alkalinity (A_T) were measured while other parameters were

479 calculated. pCO_2, CO_2 partial pressure; C_T, dissolved inorganic carbon; Ω_c and Ω_a, saturation state of

480 seawater with respect to calcite and aragonite.

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Treatment	pH_T (total scale)	A_T (mmol kg^{-1})	pCO_2 (uatm)	CO_2 (mmol kg^{-1})	CO_3^{2-} (mmol kg^{-1})	HCO_3^- (mmol kg^{-1})	C_T (mmol kg^{-1})	Ω_c	Ω_a
400 T	8.08 ± 0.00	2.516 ± 0.004	397 ± 2	0.014 ± 0.000	0.226 ± 0.001	1.974 ± 0.003	2.213 ± 0.002	5.26 ± 0.03	3.41 ± 0.02
400 T+3	8.05 ± 0.00	2.519 ± 0.004	436 ± 3	0.014 ± 0.000	0.233 ± 0.001	1.962 ± 0.004	2.208 ± 0.002	5.43 ± 0.03	3.55 ± 0.02
700 T	7.87 ± 0.00	2.517 ± 0.004	703 ± 3	0.024 ± 0.000	0.152 ± 0.001	2.155 ± 0.003	2.331 ± 0.002	3.54 ± 0.03	2.30 ± 0.02
700 T+3	7.85 ± 0.00	2.523 ± 0.004	753 ± 3	0.024 ± 0.000	0.159 ± 0.001	2.144 ± 0.004	2.326 ± 0.003	3.72 ± 0.03	2.43 ± 0.02

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486 **Table 2** ANOVA testing the effect of pCO_2 and temperature on skeletal mol% $MgCO_3$ in (A) new crusts,

487 (B) main thalli, and (C) dissolution chips of *Lithophyllum cabiochae.*

Source	df	MS	*F*	p
A) New crusts				

pCO_2	1	0.000005	0.223	0.65
Temperature	1	0.000701	28.620	**<0.0001**
pCO_2 × temperature	1	0.000011	0.444	0.51
Error	28	0.000024		
B) Main thalli				
pCO_2	1	0.000014	0.601	0.44
Temperature	1	0.000048	2.094	0.16
pCO_2 × temperature	1	0.000042	1.844	0.19
Error	28	0.000023		
C) Dissolution chips				
pCO_2	1	0.000003	0.143	0.71
Temperature	1	0.000005	0.218	0.64
pCO_2 × temperature	1	0.000014	0.663	0.42
Error	28	0.000021		

Table 3 ANOVA testing the effect of pCO_2 and temperature on difference in asymmetry mol% $MgCO_3$ in (A) main thalli and (B) dissolution chips of *Lithophyllum cabiochae.*

Source	df	MS	*F*	p
A) Main thalli				
pCO_2	1	0.000008	0.569	0.46
temperature	1	0.000006	0.441	0.51
pCO_2 × temperature	1	0.000007	0.489	0.49
Error	28	0.000013		

B) Dissolution chips				
pCO_2	1	0.000022	3.871	0.06
temperature	1	0.000001	0.190	0.67
pCO_2 × temperature	1	0.000005	0.944	0.34
Error	28	0.000006		