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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 organisms the 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 calcifying medium against ocean acidification, 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

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1. INTRODUCTION

Coralline algae are thought to be among the organisms most vulnerable to ocean acidification (decreasing pH and increasing $p\mathrm{CO}_2$). This is because their skeletons are formed 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 and aragonite (Andersson et al., 2008). Consequently, it has been suggested that coralline algae 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 $p\mathrm{CO}_2$ and temperature, and none on temperate crustose coralline algae (CCA).

The response of dead CCA crust to differing dissolution 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, higher phases of Mg-calcite 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 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 $p\mathrm{CO}_2$ than under ambient $p\mathrm{CO}_2$ (Martin and Gattuso 2009). These dissolution chip samples offer an opportunity to test the theory that higher Mg phases of Mg-calcite would dissolve preferentially 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 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 $p\mathrm{CO_2}$, and 3/ the Mg content of dead dissolution chips would decrease with elevated $p\mathrm{CO_2}$.

2. MATERIALS AND METHODS

Full experimental details, carbonate chemistry, growth 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 $p\mathrm{CO_2}$ (*ca.* 400 µatm) and ambient temperature (*T*, *i.e.* the temperature at 25 m depth in the Bay of Villefranche; control, labelled *400 T*),

(2) ambient $p\mathrm{CO_2}$ and elevated temperature (*T+3*°C; *400 T+3*),

(3) elevated $p\mathrm{CO_2}$ (*ca.* 700 µatm) and ambient temperature (*700 T*),

(4) elevated $p\mathrm{CO_2}$ 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 $p\mathrm{CO_2}$ was adjusted by bubbling ambient air (ambient $p\mathrm{CO_2}$) or $\mathrm{CO_2}$-enriched air (elevated $p\mathrm{CO_2}$) obtained by mixing pure $\mathrm{CO_2}$ 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 $\mathrm{m^{-2}\ s^{-1}}$. The photoperiod was adjusted weekly according to natural fluctuations and varied from 9:15 (Light:Dark ratio) to 15:9. The annual means of the carbonate chemistry parameters are shown in Table 1.At the end of the experiment all crusts were air-dried.

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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 original thalli (Fig. 1), (3) pieces of dead crust that had been used for dissolution tests and (4) the pink surficial crust on the original thalli (this 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) For the new crusts, sets of 4-5 crust fragments similar in size (*ca*. 2-3 mm in diameter) and thickness (~ 1 mm in diameter) , 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. Subsamples ~2-3 mm thick were cut off the sides of the original thalli and dissolution chips. The pink surface of the original thalli was sampled by gently scraping with a razor ensuring not to scrape into the white crust underneath. Scrapings from 5 algae from each treatment were required in order to obtain enough material for one XRD test.

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 added as an internal standard. Mg-content of calcite was calculated from the (104) peak position 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). XRD measurements had a reproducibility of ± 0.11 mol% (standard deviation; n=3).

The effect 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 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 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 vs 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

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Results obtained on the new crust demonstrate that the mineralogy of *L. cabiochae* is primarily controlled by temperature and scarcely constrained by $p\mathrm{CO_2}$. Similarly, the Mg content does not respond to $p\mathrm{CO_2}$ 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 $p\mathrm{CO_2}$ 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% $\mathrm{MgCO_3}$, which is consistent with the values reported in the literature, both experimentally and *in situ,* ranging between 0.4 and 2 mol% $\mathrm{MgCO_3}$ 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, $p\mathrm{CO_2}$ did not drive significant mineralogical change in living *L. cabiochae*.

The lower Mg content recorded for the pink surficial crust relative to the bulk crust is in agreement with previous studies (Diaz-Pulido et al., 2014; Nash et al., 2015). The pink surficial crust also trended up with temperature 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).

Analyses of the pre-existing thalli (main thalli) provide a baseline Mg content for *L. cabiochae*. The average across treatments was 16.1 mol% $\mathrm{MgCO_3}$, excluding the 700T treatment. This Mg content is higher than that of the new crusts grown under ambient temperatures (400T and 700T). This 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% $\mathrm{MgCO_3}$. 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.

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.

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 Mg content although this hypothesis has not been tested yet. Statistical results showed lower asymmetry for the dissolution chips compared to the main thallus. This indicates that the relative proportion of higher-Mg-phase Mg-calcite was less in the dissolution chips suggesting that the higher-Mg-phase, while still present, had suffered greater dissolution relative to the lower-Mg-phases. Dissolution experiments have demonstrated that the inter-filament Mg-calcite is the first to dissolve in pH 8 (NBS) after 1 h (Nash et al., 2013 NCC) and the cell walls remain intact until exposed to pH 7.7-7.82 over several hours. The pH in the present experiment did not drop below pH_T 7.8 in the 700T or 700T+3 treatments (Martin and Gattuso, 2009). Considering these previous studies and the data presented here, it seems likely that the cell walls have remained substantially intact but the inter-filament Mg-calcite has remineralized to a lower phase of Mg-calcite and there may also be abiotic Mg-calcite infilling cell spaces prior to complete dissolution of the exposed edge. The process of cell infill by Mg-calcite has been observed in the exposed bases of tropical CCA *P. onkodes* (Nash et al., 2013a) whereby exposed dead cells are in-filled with Mg-C. XRD analyses of the exposed base of the tropical CCA measured 14.8 mol%

$MgCO_3$ compared to the main crust of 16.9 mol% $MgCO_3$ (Nash et al., 2013b) indicating that the abiotic Mg-calcite has lower average Mg content than the original crust.

If the proposal for remineralization of the dissolution chips is correct, then the results for the present study would indicate that there is no trend with Mg and temperature or CO_2 for abiotic mineral formation. This would be in contrast to results for synthetic formation of Mg-calcite (Mucci 1987) although the trend for synthetic Mg content was substantially less than uptake for biogenic Mg-calcite, with an increase of only 2 mol% $MgCO_3$ from 6 to 8 mol% $MgCO_3$ from 5 to 25°C. Support is provided for the absence of temperature trend by another comparison of the results for the dissolution chips to dead tropical CCA sampled from a coral reef core from Rodrigues Island, Indian Ocean (Rees et al., 2005) where the Mg content of the dead crusts was 15 to 15.3 mol% $MgCO_3$ (Nash et al., 2013b). To thoroughly test the hypothesis for an absence of temperature trend in abiotic Mg-calcite mineralization, a comprehensive survey of dead CCA from a range of latitudes would be required. However, the clear trend for increase in Mg uptake by living CCA as temperature increases, compared to the absence of trend in altered dissolution chips, suggests the Mg content increase may be primarily driven by a biological response, rather than abiotic thermodynamics alone that the organism is unable to compensate for as suggested by Diaz-Pulido et al. (2014).

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), 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 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). 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 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). 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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Authors declare no existing competing financial interests in this work.

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Figures

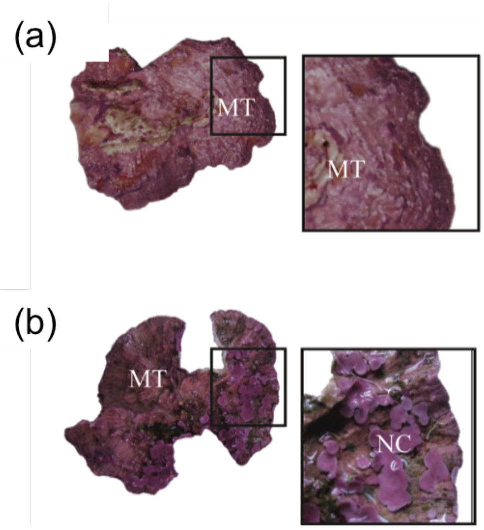

Figure 1: Bottom face of the main thallus (MT) of *L. cabiochae* (a) free of crusts at the time of collection and (b) with new crusts (NC) grown during the experimental period.

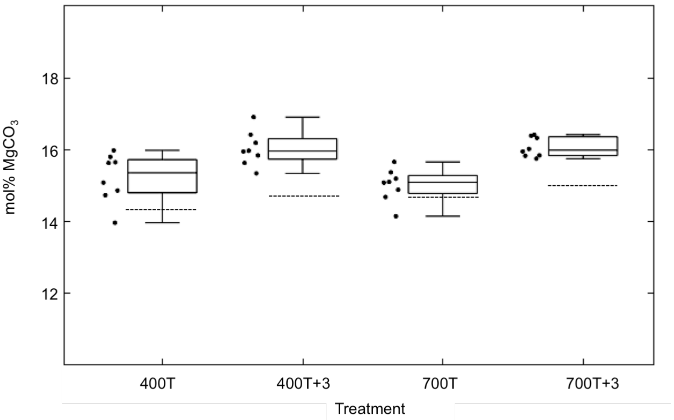

Figure 2: XRD results for new crust and pink surficial growth from the 400 and 700 µatm treatments, in ambient temperature and ambient + 3 °C. Dashed lines shown the mean mol% MgCO$_3$ for pink surficial growth. The box plots represent the new crust and the dots are individual data points. The boxes represent 25 and 75 percentiles, the horizontal bold line is the median value and the whiskers are minimum and maximum values.

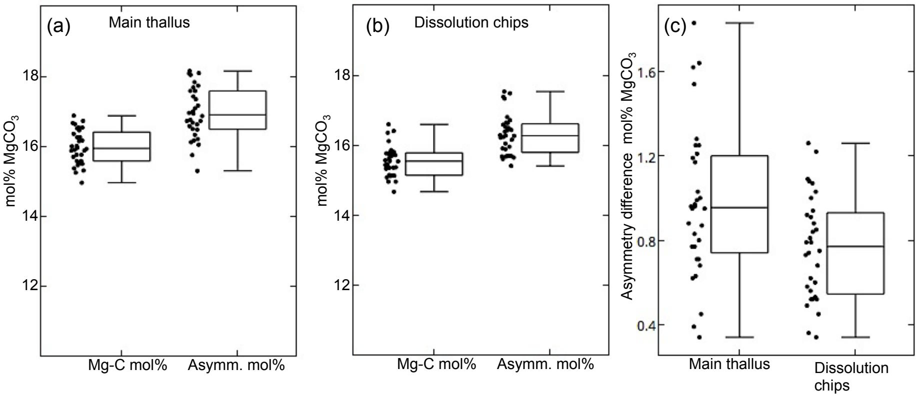

Figure 1: XRD results for main thalli and dissolution chips. **(a)**. Mol% MgCO$_3$ and asymmetry mol% MgCO$_3$ for the main thallus. **(b)**. Mol% MgCO$_3$ and asymmetry mol% MgCO$_3$ for the dissolution chips. **(c)**. Difference in asymmetry mol% MgCO$_3$ between the main thallus and dissolution chips indicating a reduction in the higher phases of Mg-calcite after dissolution.

Table 1 Parameters of the carbonate system in each treatment.

The values reported are means (± standard error) of 191 to 194 data collected from July 2006 to August 2007. The pH (pH_T, on the total scale) and total alkalinity (A_T) were measured while other parameters were calculated. pCO_2, CO_2 partial pressure; C_T, dissolved inorganic carbon; Ω_c and Ω_a, saturation state of seawater with respect to calcite and aragonite.

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

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

$p\mathrm{CO}_2$	1	0.000014	0.601	0.44
Temperature	1	0.000048	2.094	0.16
$p\mathrm{CO}_2$ × temperature	1	0.000042	1.844	0.19
Error	28	0.000023		
C) Dissolution chips				
$p\mathrm{CO}_2$	1	0.000003	0.143	0.71
Temperature	1	0.000005	0.218	0.64
$p\mathrm{CO}_2$ × temperature	1	0.000014	0.663	0.42
Error	28	0.000021		

Table 3 ANOVA testing the effect of $p\mathrm{CO}_2$ 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				
$p\mathrm{CO}_2$	1	0.000008	0.569	0.46
temperature	1	0.000006	0.441	0.51
$p\mathrm{CO}_2$ × temperature	1	0.000007	0.489	0.49
Error	28	0.000013		
B) Dissolution chips				
$p\mathrm{CO}_2$	1	0.000022	3.871	0.06
temperature	1	0.000001	0.190	0.67
$p\mathrm{CO}_2$ × temperature	1	0.000005	0.944	0.34
Error	28	0.000006		