Response to reviewers for manuscript

Ocean acidification does not affect magnesium composition or dolomite formation in living crustose coralline algae, Porolithon onkodes in an experimental system

M. C. Nash, S. Uthicke, A. P. Negri, and N. E. Cantin

We thank both reviewers for their positive comments and recommendation for publication in Biogeosciences. We address their specific comments and edits below.

Reviewer 1

The authors repeatedly talk about a pink and white crust, and sometimes also about ?old growth as a white crust? and ?new white crust?. Only once, at the beginning of the discussion, they relate the above terms to more standard coralline algal terminology (epithallus the actively calcifying pigmented uppermost layer; and perithallus the underlaying calcified structure). This can be confusing to the reader and I would suggest to use the algal terminology throughout, and label it either on figure 1 (by the way, this figure doesn't have a scale) or on an adjacent drawing.

Action-A paragraph defining the terminology used and linking to standard coralline terminology has been added after the methods. Figure 1 has been relabeled. Scale has been added to figure 1.

….“Crust terminology- The term 'pre-experimental growth' refers to crust grown in situ at Davies reef prior to collection for the experiment. The new crust (experimental) is the growth above the height of the resin. The 'new crust' terminology is used because this includes both the white crust of the perithallus and the pink surface epithallus. There may also be regrowths within the white crust that include hypothallus cells and alteration to aragonite (see for example Fig. 8). The new settlement on slides in the 6 month treatment was predominantly pink indicating epithallus growth. However when CCA settle, the first cells laid down are hypothallus cells growing lengthways parallel to the surface and then vertical growth of the epithallus, followed by the perithallus (Steneck 1986) a scraping sample would include not only epithallus but also minor hypothallus and possibly the start of a perithallus. For this reason we use the term new settlement rather than epithallus. “
Also the authors should add a brief description on how the white crust is formed (e.g. calcification at the meristem? or from the pink crust? after it loses its pigmentation? Does the pink crust commonly get grazed down or is it subject to what has been referred to as epithallial sloughing (e.g. will it flake off?).

Action-This description has been added to the introduction, 3rd paragraph and extra references added.

…..“The surface of the CCA is the epithallus. The pink colouration is due to the presence of pigmented photosynthetic tissue within the Mg-calcite skeleton. In other species of corallines, this pink surface has been shown to slough off (Pueschel et al. 2005) and be grazed by chitons and limpets (Adey et al. 2013). The white crust underneath (perithallus) has been shown in other species of CCA to form as cell by cell growth downward from the meristem cells (growth layer between epithallus and perithallus) (Adey et al. 2013). Thus the white crust is a product of meristem growth, and not a build up of epithallus growth after it loses its pigmentation.”


Also the distinction of old and new white crusts is a bit unclear, I assume it refers to pre-experimental and experimentally formed crust. If so, how can you distinguish them (by the way the saw cut? or did you apply some kind of a stain at the initiation of the experiment?).

Action-This has been addressed on the new terminology paragraph and old has been changed throughout the MS to pre-experimental. Also, it has been noted in the MS that identification of the new crust was by the overgrowth on the resin (terminology paragraph and in methods already) and by following the line of this growth across (legend to Fig. 6). Labeling on figure 1 has been changed to better identify the different portions of CCA crust.
…”Transition from pre-experimental growth to experimental identified by following the growth lines from the crust on the resin (not pictured) across the sample”.

Typos: p. 1374 Line 12 settlement was affected p. 1385 Line 21- is not abundant p. 1390 Line 9 - mean

Action-Edits made

Reviewer 2

Some of the figure legends have inference in them. I would leave that in the results discussion sections.

Action-Removed

P1384 L 24: could this be post depositional change?

Good question and one I have considered often. The following paragraph has been added to the discussion with additional references added.

Considering that CCA crusts are increasingly being used for paleo environmental reconstruction (e.g. Kamenos et al. 2008, Halfar et al. 2013, Caragnano et al. 2014, Darrenougue et al. 2014, Fietzke et al. 2015), it is important to know whether this difference in magnesium composition between the pigment surface and white crust is part of the standard calcification processes of the CCA or due to post-depositional change. In this and previous work (Nash et al. 2011, 2013a) portions of the crust that have been diagenetically altered post-deposition have
cells in-filled by aragonite or Mg-calcite. Typically the cell walls have not exhibited evidence of alteration even when there has clearly been exposure to seawater suggesting the intact cell walls are quite resistant to diagenesis. Probably the epithallus cell walls and perithallus cell walls have differences in the organic material that constrains the Mg uptake. The interfilament and intrafilament (spaces between adjacent cell walls) calcification does not appear to be physically constrained by an organic template with crystals randomly orientated (Nash et al. 2013a, Adey et al. 2013) or roughly parallel to the cell walls which suggests that the controls on calcification and consequently Mg incorporation may be different again for the interfilament calcification. It seems most likely that the difference in the mol% MgCO$_3$ for the white crust compared to the pigmented new growth is due to organism-constrained Mg uptake during the crust development. It cannot be determined from this study whether the Mg is incorporated in its final concentrations as the new cell wall and inter/intra filament calcification is first formed or if there is subsequent Mg enrichment over days/weeks/months. However, previous work subsampling portions of the CCA crust from the top to the base has not demonstrated any systematic increase in mol% MgCO$_3$ (Nash et al. 2013b) suggesting if there is post-deposition Mg enrichment, it occurs relatively contemporaneously with growth.


P1386 L10: also contrast to Kamenos et al 2013 GCB which show a fall in Mg concentrations of L glaciale at high pCO2.

Action- Reference to the results for the living, relevant to this experiment, has been added.
"Lithothamnion glaciale showed no change in [Mg] for new growth over 80 days in reduced pH 7.7 treatments (Kamenos et al. 2013)."

P1386 L16: All the references are for free living or articulated species rather than crusts. I would reword to “coralline algae structure” and make it complete also add Kamenos et al 2013 Glob Ch Biol and Burdett et al 2012 Mar Biol Res 8:756-763 to the list both of which consider structure (skeletal and epithelial correspondingly).

Action- Edits made

P1389 L20: Not sure you can make that conclusion about the organic film as you have not measured photosynthesis etc. Also, from the micrograph it could be an artefact. I would remove this sentence to be on the safe side.

*Photosynthesis would have to have been measured in the open environment first to obtain a natural baseline and then compared to photosynthesis in the control tank CCA. This comparison was made by Chisholm 2003 (already referenced and discussed lines 15-18) and showed that photosynthesis and production of inorganic carbon (calcification) and organic carbon was less in tanks than in situ in the natural environment. Thus his results support our conclusions here. We do not think the absence of an organic film is purely an artifact of preparation.*

Action- Sentences added to justify this conclusion.

…”This organic film is consistently present on the pre-experimental growth and consistently absent from the experimental growth. Thus it is unlikely to be a sample preparation artifact, although the preparation method may make this film more readily visible than if the samples had been fractured leaving an uneven surface.”

Pg 1374 L12: “was affected”

Action- changed.

Pg 1374 L14: “affected” rather than “effected” and “affect” rather than “effect”

Action- changed

Pg 1375 L20: Rhodoliths
Action- changed

Pg 1377 L14 and through out. Refer to the pCO2 as uatm. Strictly, ppm is atmospheric CO2.

Action- changes made

Pg 1378 Section 2.3. First sentence is quite long, could be made into two smaller ones.
Action- changed (although it was the 3rd sentence that was long, and partially repetitive).

Pg 1380 L3. Space between P and onkodes. Check this through out, there are a few places where space is missing.
Action- checked and changed throughout MS

Pg 1380 L67 Mastigocoleus should have a sp. after it and be in italics
Action- changed

Pg 1382 L1 “have been” should be changed to “were” to keep the tenses the same
Action- changed

Pg 1387 L13: Therefore - add an e at the end
Action- changed

Pg 1388 L25: Therefore - add an e at the end
Action- changed

Table 1: give the same number of decimal places in all. Also if p < 0.001 just quote p < 0.001.

Figure 2: Very clear figure. Just one comment; change the star symbol to a triangle or square as stars have a particular statistical meaning (indicate statistical significance at p < 0.05).
Action- changed

Figure 4: There are two scale bars on the micrographs, I would remove the instrumentally added one and just use the bar you have added, this will make the micrographs less busy. I would also make the text larger so it is easier to see. This applies to all the figures with micrographs in them

Action – changed for all figures

Figure 5: As with figure 4. Also for c) The dashed arrow seems to be pointing to “SEM charging” captured in the micrograph. Also, is the organic covering due to sample preparation if it is on the exposed surface?

Action- arrow shifted, organic film and sample preparation addressed in previous edit.

Figure 6: A little hard to work out what is going on here due the saw damage on the sample. How do you know what is pre experimental growth and also what is dolomite infill? Do you have micrographs similar to those in Fig 7?

Yes-there are images similar to those in Fig in the supplementary information, Fig 3, referred to at line 24, page 1383.

Action- Extra note added to legend to Fig 6 to refer again to SI Fig. 3

Action- text edited to note how experimental growth is identified- by following growth line from resin over pre-experimental crust.

Dolomite infill identified by EDS measurements (listed in SI) within the range of 38-62 and by the grey scale. Text added to Fig 4 legend to explain how backscatter SEM is used to identify dolomite

…”Backscatter SEM shows the lighter elements i.e. magnesium, as darker gray and heavier elements, i.e. calcium are pale gray to white whereas secondary electron images shows the topography of the sample but does not provide information on the elemental composition. EDS measurements are made in the different grade shade areas to measure
Mg composition (range listed SI) and this is used to identify the mineral composition. Once the measurements have been made it is possible then to identify dolomite and calcite from the grey shade.”

Figure 8: Define A and B in (a) and (b). (d) is indicated as D in the legend. Scale bar sentence should be (b), (c), (d) so not to confuse with D for dolomite.

*Action- definitions added and edits made*
Ocean acidification does not affect magnesium composition or dolomite formation in living crustose coralline algae, *Porolithon onkodes* in an experimental system

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Abstract

There are concerns that Mg-calcite crustose coralline algae (CCA), which are key reef builders on coral reefs, will be most susceptible to increased rates of dissolution under higher $pCO_2$ and ocean acidification. Due to the higher solubility of Mg-calcite, it has been hypothesized that magnesium concentrations in CCA Mg-calcite will decrease as the ocean acidifies, and that this decrease will make their skeletons more chemically stable. In addition to Mg-calcite, CCA *Porolithon onkodes* the predominant encrusting species on tropical reefs, can have dolomite ($Ca_{0.5}Mg_{0.5}CO_3$) infilling cell spaces which increases their stability. However, nothing is known about how bio-mineralised dolomite formation responds to higher $pCO_2$. Using *P. onkodes* grown for 3 and 6 months in tank experiments, we aimed to determine (1) if mol % MgCO$_3$ in new crust and new settlement affected by increasing $pCO_2$ levels (365, 444, 676 and 904 ppm), (2) whether bio-mineralised dolomite formed within these time frames, and (3) if so, whether this was effected by $pCO_2$. Our results show there was no significant effect of $pCO_2$ on mol % MgCO$_3$ in any sample set, indicating an absence of a plastic response under a wide range of experimental conditions. Dolomite within the CCA cells formed within 3 months and dolomite abundance did not vary significantly with $pCO_2$ treatment. While evidence mounts that climate change will impact many sensitive coral and CCA species, the results from this study indicate that reef-building *P. onkodes* will continue to form stabilising dolomite infill under near-future acidification conditions, thereby retaining its higher resistance to dissolution.

1 Introduction

Determining the influence of ocean acidification, due to increasing atmospheric CO$_2$ concentrations, on mineral formation of crustose coralline algae is not only important to understand potential changes in CCA and their reef building capacity into the future, but also to understand the past. As atmospheric carbon dioxide (CO$_2$) concentra-
tions increase, fundamental changes to the ocean’s chemistry follow. Seawater pH and the carbonate saturation state (Ω) decreases, thus increasing the solubility of CaCO₃ skeletons. Current projections are that by the end of this century, if anthropogenic CO₂ emissions continue unabated, tropical surface seawater pH will drop by 0.3–0.4 units to ~ pH 7.8 (Orr, 2011). Marine organisms forming carbonate skeletons are susceptible to increased rates of dissolution as pH declines (reviewed in Howard et al., 2012). There are concerns that Mg-calcite crustose coralline algae (CCA) will be one of the first reef-building organisms to suffer as CO₂ rises (e.g. Diaz-Pulido et al., 2012), due to the higher solubility of their skeleton. The possibility has also been raised that CCA may decrease their uptake of magnesium to form more stable lower Mg-calcite in response to higher CO₂ concentrations (e.g. Andersson et al., 2008; Ries, 2011).

Experimental data on the impacts of pH on magnesium uptake by tropical CCA are limited. The branching coralline Neogoniolithon demonstrated a decreased magnesium concentration in experimental severely low pH conditions (Ries, 2011). However, CCA Porolithon onkodes transplanted into low pH treatments for 8 weeks did not exhibit any magnesium composition change with pH in new surface tissue (Diaz-Pulido et al., 2014). Temperate coralline Corallina elongate had a variable response with new growth on existing branches not exhibiting a response to elevated CO₂ whereas new structures grown during the experiment did have decreased Mg content in higher CO₂ treatments (Egilsdottir et al., 2012). Temperate rhodolith Lithothamnion glaciale did not change Mg content in different CO₂ treatments while living, however a significant decrease in the Mg content in low pH compared to dead thalli in the same treatment raised the possibility that there was a biological response (Kamenos et al., 2013). Recently it was discovered that tropical CCA P. onkodes commonly possess additional magnesium minerals dolomite (Mg₀.₅Ca₀.₅CO₃) and magnesite (MgCO₃) infilling cells in the crust (Nash et al., 2011). This additional mineralisation significantly reduces rates of skeletal dissolution compared to P. onkodes without dolomite cell infill (Nash et al., 2013a). A combination of high CO₂ and increased temperature over 8 weeks led to a ~ 300 % increase in the relative quantity of dolomite in P. onkodes crust
transplanted into the treatment conditions (Diaz-Pulido et al., 2014). This was due to endolithic cyanobacteria, *Mastigocoleus* removing calcium from the Mg-calcite skeleton but not from dolomite, leading to destruction of Mg-calcite and a relative increase in dolomite. It could not be determined if there was also an increase in the formation of primary dolomite. When CCA grow to form the thick crust crucial to cementing together the structural reef framework, the new growth extends upwards leaving the old growth as a white crust without pink photosynthetic pigment is in this important reef-structure forming white crust that dolomite infill is abundant (Nash et al., 2011; Diaz-Pulido et al., 2014). As yet, there have been no experiments to determine the impact of CO$_2$ levels on mol% MgCO$_3$ and dolomite formation in the white crust grown in differing CO$_2$ treatments.

There is a noted correlation of dolomite abundance and greenhouse conditions (high temperature, high CO$_2$) over the geological past (e.g. MacKenzie et al., 2008; Wilson and Given, 1986). To understand the past, it is necessary to separate the roles that CO$_2$ and temperature may have had on constraining dolomite concentration. This study describes the first experiments that constrain the role of CO$_2$ on a biomineralised dolomite formed in differing CO$_2$ environments.

The aims of this investigation were threefold, (1) to identify any changes in mol% MgCO$_3$ in new settlement and new white crust of *P. onkodes* grown in pre-industrial, Control (present day), Medium (near future) and High (end of century) CO$_2$ (IPCC, 2007) conditions over 3 and 6 months, (2) to determine whether bio-mineralised dolomite is formed within these timeframes, (3) to determine if the CO$_2$ concentration affects bio-mineralised dolomite formation.
2 Methods

2.1 Experiment

Fragments of live *P. onkodes* were collected from the upper reef crests (2–3 m depth) of Davies Reef (18°49.29′ S, 147°37.99′ E), Great Barrier Reef in August 2012. To eliminate open carbonate surfaces, CCA chips (~1 cm diameter) were sealed around the sides and base in non-toxic under water glue (Mr. Sticky’s, Fair Oaks, CA) and attached to PVC slides (only the top live surfaces were exposed to seawater). Blank slides were also added to the system to identify and track new CCA settlement. Slides were mounted in custom perspex holders which were held in place on aquarium walls using magnets. The experimental system used was described in (Uthicke et al., 2013).

Briefly, fresh filtered seawater (0.4 mm) was added to three replicate tanks (for each treatment) replacing the water twice daily. Flow rates in each experimental tank were 12 L min⁻¹. In addition to a present day (pHₜ 8.0 target, measured mean 7.96 ± 0.04 SE CO₂: 444 ± 37 ppm), mid century 2050 (future pHₜ 7.9 target, measured mean 7.90 ± 0.04 SE CO₂: 676 ± 37 ppm) and end of century 2100 (future pHₜ 7.75 target, measured mean 7.77 ± 0.06 SE CO₂ 904 ± 32 ppm) target acidification treatments, this experiment also included a pre-industrial treatment (past pHₜ 8.14 target, measured mean 8.09 ± 0.04 SE CO₂: 365 ± 37 ppm). Acidified treatments were achieved by bubbling CO₂ into sump tanks with solenoid valves (SMC pneumatics) and pH setpoints, while the pre-industrial treatment was achieved by passing a stream of air through 2 soda lime canisters and mixing the low CO₂ scrubbed air with the incoming seawater in a counter current exchange tower prior to flowing into each experimental tank. Temperatures were controlled (Avg. 26.1 ± 0.15 °C) with a heater chiller unit (EvoHeat DHP40). pH and temperature were monitored continuously (30 s sampling rate) with IS-FET type pH probes (Endress Hauser CPS-471D). Seawater CO₂ concentrations were measured using a LiCor (LI-840A) CO₂/H₂O analyser. This experiment was conducted within the outdoor aquarium facility at the Australian Institute of Marine Science under natural daily light cycles during the Austral summer (October–April). Outdoor light in-
tensities were reduced with 70% UV blocking green shade cloth to an average intensity of 210 ± 12 µmol photons m⁻² s⁻¹, with a daily maximum of 330 µmol photons m⁻² s⁻¹. These light intensities correspond to the daily average light intensity on shallow reefs.

2.2 Sample selection

Subsets of CCA’s in resin were removed from the tanks after 3 and 6 months. The settlement slides were removed after 6 months. Samples were randomly selected from these for XRD analyses. New crust from the resin-embedded CCA’s was sampled by breaking off crust that overgrew the resin. This ensured that only crust formed during the experiment was included in the new crust analyses. The new crust typically had a thin layer (~ 0.5 to 2 mm) of white crust overlain by a layer of pink photosynthetic tissue/crust (Fig. 1). CCA that had settled on the plastic slides after 6 months had only pink crust and there was no white crust underneath. Typically for the new settlement CCA, 2–4 settlement patches were required to obtain sufficient material for analysis by XRD, thus each individual result for new settlement is an average of several CCA patches. These CCA had not reached reproductive stage and could not be identified. For the 6 month experiment, CCA’s in resin from the control tanks were unavailable for mineral analysis.

2.3 Analyses

CCA were cut using a bench-top saw with a 2 mm thick diamond impregnated blade. A slice through the middle of each 3 month sample was kept for SEM. CCA were carbon coated and Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS) was undertaken at the Australian National University using a Ziess UltraPlus field emission scanning electron microscope (FESEM) equipped with an HKL electron backscatter diffraction (EBSD) operated at 15 kV, 11 mm working distance. CCA were mounted using carbon tape and carbon coated. Subsampling for XRD was taken from the matching side of the remainder crust. Xray diffraction and mineral determination
was carried out following Nash et al. (2013b). Simply, this method uses the asymmetry off the higher 2-theta side of the Mg-calcite XRD peak to detect dolomite. The more asymmetry the greater proportion of dolomite in the crust. A shoulder off the higher 2-theta side of the peak indicates magnesite (MgCO$_3$) is also present. This asymmetry and shoulder is captured with the asymmetry mol% measurement. The asymmetry mol% is used to compare for differences in relative dolomite and magnesite quantities (Nash et al., 2013b). It is not a measurement of absolute quantity, however when compared to mineral quantities determined using standard curve fitting techniques, the differences in asymmetry well reflect the differences in dolomite and magnesite quantities (as used in Diaz-Pulido et al., 2014). See Fig. 1 (Supplement) for example scans.

### 2.4 Dolomite terminology

Stoichiometric dolomite is 50 mol% MgCO$_3$. Typically dolomite formed under high temperature is stoichiometric and well ordered (Kaczmarek and Sibley, 2011). Ordering occurs where there are alternating layers of MgCO$_3$ and CaCO$_3$ in the calcite lattice, whereas completely disordered dolomite has Mg randomly substituting for Ca in the lattice. Sedimentary dolomite formed at sea surface temperature and pressure and not subject to post-deposition burial and metamorphism, typically is non-stoichiometric with a range of 37.5 to 52 mol% MgCO$_3$ (Jones et al., 2001) and not well ordered (Kaczmarek and Sibley, 2011). Synthetically formed disordered dolomite has been shown to be unstable in aqueous solutions and therefor it is thought that disordered dolomite cannot form or persist in the open marine environment in which sedimentary dolomite forms (Gaines, 1977). A variety of descriptions exist for dolomite that deviates from stoichiometric and perfectly ordered; non-ideal, poorly ordered or disordered, protodolomite, pseudo-dolomite and calcium enriched dolomite (Gaines, 1977).

Here we use the term dolomite to represent magnesium calcite in the range 38–62 mol% MgCO$_3$, as measured for CCA *P. onkodes* dolomite (Nash et al., 2011) without inferring cation ordering status, that is, whether it is ordered, disordered or partially ordered. The CCA dolomite has previously been demonstrated via etching experiments.
and natural dissolution processes to have a delayed dissolution reaction compared to Mg-calcite and has different crystal forms to Mg-calcite (Nash et al., 2013a). Furthermore, it has been documented that Mg-calcite in CCA *Ponkodes* ranges up to \( \sim 26 \text{ mol\% MgCO}_3 \) (Nash et al., 2011) and there is a well-defined division from dolomite which commences at \( \sim 38 \text{ mol\% MgCO}_3 \). Experimental work has demonstrated that cyanobacteria (Mastigocoleus) which bio-erode limestone by removing calcium, do not take calcium from dolomite rock (Ramirez-Reinat and Garcia-Pichel, 2012). Experiments on live dolomite-forming CCA *Ponkodes* also show that the same cyanobacteria remove calcium from CCA Mg-calcite but do not remove calcium from the *Ponkodes* dolomite. *Ponkodes* Mg-C and *Ponkodes* dolomite have distinctly different physical properties and *Ponkodes*-dolomite reacts under chemical (Nash et al., 2013a) and bio-erosion conditions (Diaz-Pulido et al., 2014) comparably to dolomite the rock. We have been unable to confirm the presence of ordering peaks by XRD for the dolomite within the living *P. onkodes* (Nash et al., 2013b). However the persistence of the CCA dolomite in aqueous environments and its greater resistance to dissolution than Mg-calcite (Nash et al., 2013a) suggests there is some degree of ordering and CCA dolomite is not the same mineral as Mg-calcite which theoretically becomes less stable with greater Mg-substitution (Andersson et al., 2008). Therefore we consider that referring to the CCA mineral as dolomite, with the caveat that this is without inferring cation-ordering status is the most appropriate identification for the mineral at this time. Our decision to use this terminology for Mg-C > 38 mol\% MgCO\(_3\) is supported by recently published clarification on terminology for Ca-Mg carbonates (Zhang et al., 2015).

### 2.5 Statistical analysis

We tested for difference between \( pCO_2 \) treatments and sample type using two factor analysis of variance (ANOVA). Different CO\(_2\) treatments (Factor Treatment) and experimental growth vs. pre-experimental growth (Factor Type) were both used as fixed factors. Residual plots and boxplots confirmed that there were no deviations from ANOVA
assumptions. Because slightly unequal sample sizes were used in each treatment, we applied marginal sums of squares for the $F$ tests.

3 Results

3.1 Mineral composition in different CO$_2$ treatments

We investigated the mineral composition of $P$. onkodes exposed to different OA conditions for 3 and 6 months in a long-term aquarium experiment. There were no significant differences in mineral composition between any of the CO$_2$ treatments (Table 1). For the new $P$. onkodes crust formed during the 3 month duration (Fig. 2a), the mol% MgCO$_3$ range was 16.4–16.7 mol% MgCO$_3$ ($n = 5$ per treatment, averages: Pre 16.6, Control 16.5, Medium 16.4, High 16.7 mol% MgCO$_3$) (full results Supplement Table 1). This range was only 0.1 mol% more than measurement precision (Nash et al., 2011). For the new $P$. onkodes crust formed over 6 months (Fig. 2b), the mol% MgCO$_3$ range was the same as the 3 month crust 16.4–16.7 mol% MgCO$_3$, (Pre 16.7 $n = 5$, Medium 16.4 $n = 3$, High 16.5 mol% MgCO$_3$ $n = 6$) (Supplement Table 2). Many of the Mg-calcite XRD peaks for both the 3 and 6 month crust demonstrated asymmetry indicating the presence of dolomite (as per Nash et al., 2011, 2013a, b; Diaz-Pulido et al., 2014) however there was no significant difference in the dolomite asymmetry related to CO$_2$ treatments (asymmetry test, Table 1). For unidentified CCA that had settled on the slides over 6 months (Fig. 2c), (Supplement Table 3) the mol% MgCO$_3$ ranged from 14.7–14.9 (Pre 14.8 $n = 3$, Control $n = 4$ 14.7, Medium 14.7 $n = 5$, High 14.9 mol% MgCO$_3$ $n = 5$). The new settlement CCA did not have dolomite, i.e. no peak asymmetry, consistent with the absence of white crust underneath.
3.2 Mineral compositional differences between crust layers

As there was no significant difference between treatments, all treatments have been combined for each time period. There was a significant difference in magnesium composition between experimental crust and pre-experimental crust. Mg-calcite mol% MgCO$_3$ was also significantly different for new settlement (pigmented growth without development of white crust) compared to new crust (growth that has developed white crust). The 6 month new settlement (pigmented growth only) at 14.8 mol% MgCO$_3$ (Fig. 3) was significantly lower than the mol% MgCO$_3$ for the new crusts from the 3 and 6 months new crusts (~16.5 mol% MgCO$_3$). The asymmetry indicating dolomite presence was absent from the new growth, but appeared in new white crust within 3 months (Asymm mol% 17.6) and was higher again for the 6 month new crust (Asymm mol% 18.7). The mol% MgCO$_3$ and asymmetry mol% in the pre-experimental *P. onkodes* crust (the crust formed in the natural environment prior to sample collection) were even higher at 17.5 and 21.6 mol% MgCO$_3$ respectively (Fig. 3) (full data Supplement Table 4).

3.3 SEM results

3.3.1 Comparison of crust across treatments and experimental/pre-experimental

Although there was no detected difference in mineral composition across treatments, SEM was undertaken on a selection of the 3 month crusts to visualise potential differences in calcification structures between treatments. There was no visible difference in calcified crust detected between pre-industrial, control or high CO$_2$ treatments. There was however, a clear difference in the structure of the crust grown during the experimental duration compared to the pre-experimental crust (Figs. 4, 5, and Supplement Fig. 2). This difference was observed for control, as well as pre-industrial and high CO$_2$ crusts indicating the difference was not related to the CO$_2$ levels. Crust formed dur-
ing the experiment appeared less organized and also appeared structurally less dense (Fig. 6) with cracks and associated gaps in the crust that were not present in the pre-experimental crust. The difference in density was based on observation and not able to be quantified.

The experimental crust had compressed under the action of the saw used to slice the CCA (Fig. 7). We note that this compression by the saw would have made it difficult to identify any differences in growth structure between the CO$_2$ treatments. Previous work relying on SEM for CCA interpretation has used both saw cutting similarly to here (Nash et al., 2011, 2013a, b; Diaz-Pulido et al., 2014) as well as fracturing without any further treatment of the sample (Nash et al., 2013a; Diaz-Pulido et al., 2014). There has not been an observed impact of saw cutting on experimental samples (Diaz-Pulido et al., 2014) however those previous samples were polished after cutting and fine cracks may have been less obvious due to polishing. The crust features in the pre-experimental crust are comparable to features in other $P$. onkodes analysed using SEM that have been cut, cut and polished or only fractured (Nash et al., 2011, 2013a, b; Diaz-Pulido et al., 2014) and it is unlikely that the use of the saw has introduced an artifact into this study other than to highlight the susceptibility of the experimental crust to crushing compared to pre-experimental crust.

### 3.3.2 Dolomite features

Dolomite composition determined by SEM-EDS ranged from 37.3 to 59.8 mol% MgCO$_3$ (Table 5 Supplement), comparable to the range identified in previous studies (Nash et al., 2011). There was a delineation along the new experimental growth where dolomite was nearly absent compared to consistent infill in pre-experimental growth (Figs. 5–7, Supplement Fig. 3). The structure of dolomite formed in the experimental crust also appeared different to that which formed in the pre-experimental crust (Fig. 4). New growth dolomite did not generally fill the cells as was observed in the pre-experimental growth. In the experimental growth, dolomite was present as lumpy infill or lining (Fig. 4a and b). In the pre-experimental crust, dolomite lined and in-filled most
cells (Fig. 4c and d). In the control CCA the pre-experimental crust had an opaque organic film that was not visible in experimental growth (Fig. 5c), although there was organic material in the cells (Supplement Fig. 3).

3.3.3 Crust damage possibly due to transfer to experimental tanks

Pre-experimental crust immediately below experimental growth had aragonite cell infill (Fig. 7). In previous work aragonite infill of this type has only been observed at the base of the CCA crust exposed to seawater (Nash et al., 2013a Supplement), or in parts of the skeleton that have been damaged allowing seawater to penetrate. However, we could find no obvious signs of damage to the crust. CCA *P. onkodes* has varied mineralogy throughout the pre-experimental crust (Fig. 8) with patches altered to aragonite and dolomite bands. Regrowth in damaged areas within the pre-experimental crust was more dolomite rich than surrounding areas (Fig. 8b) indicating that damage to crust in the open environment had not resulted in a reduction in dolomite formation.

4 Discussion

Our results show that over the experimental duration (1) there were no changes in any crust mineral composition relating to CO\(_2\) concentrations, (2) bio-mineralised dolomite forms within 12 weeks within aquarium conditions; and (3) CO\(_2\) concentrations do not affect bio-mineralised dolomite formation.

4.1 Magnesium composition and calcification processes

The higher mol\% MgCO\(_3\) for white crust compared to the pigmented new growth layer (new settlement) has been documented previously for *P. onkodes* (Diaz-Pulido et al., 2014). This higher mol\% MgCO\(_3\) in the white crust suggests that controls on magnesium uptake are different for the white crust (perithallium) than the pigmented surface layers (epithallium).
The consistency of magnesium composition across *P. onkodes* and new settlement CCA from pre-industrial to high CO₂ treatments does not provide support for the theory that Mg-C organisms will take up less magnesium under higher CO₂ conditions (Andersson et al., 2008). Instead our results agree with the response of *P. onkodes* in an 8 week laboratory aquarium experiment which also showed no change in mol% MgCO₃ in pigmented growth with CO₂ levels up to 1225 µatm (Diaz-Pulido et al., 2014). Those CCA were not embedded in resin and were grown in higher temperatures (28 and 30°C). Both these aquarium experimental results are in agreement with new settlement CCA in CO₂ enriched flow through systems (Kuffner et al., 2008). This consistency of mol% MgCO₃ suggests there is a strong biological control on magnesium uptake under variable CO₂ concentrations and no detectable plastic response to CO₂ within the experimental ranges. The absence of change across treatments for mol% MgCO₃ in the new settlement CCA, none of which have dolomite, suggests that the similar apparent lack of response of the mol% MgCO₃ in the white crusts to CO₂ treatments is unrelated to the presence of dolomite. The lack of difference between pre-industrial, medium and high treatments in the 6 month crust sample set suggests that no trends have been missed with the absence of the control group.

### 4.2 Comparison to other studies

The results from the *P. onkodes* are in contrast to the decreased magnesium composition for tropical branching *Neogoniolithon* sp. (Ries, 2011). This form of *Neogoniolithon* are not abundant in the high-energy environments that *P. onkodes* dominates. However, the mol% MgCO₃ measured in the *Neogoniolithon* control (∼18.7–21.3 mol% MgCO₃) was much higher and with greater range than that measured for *P. onkodes* in this experiment (pre-experimental crust 17.2–17.9, 3 month crust 16–16.8, new settlement 14.4–15.3 mol% MgCO₃ Supplement Tables 1, 3, and 4). mol% MgCO₃ in the *Neogoniolithon* decreased to 18.7–16.7 mol% at 903 ppm CO₂ (equivalent CO₂ levels as our highest treatment) but only decreased by another 1.3 mol% MgCO₃ on average (range 17.3–16.0 mol% MgCO₃) with an extra 1962 ppm (2865 ppm CO₂). Thus
the lowest Mg levels for the *Neogoniolithon* in the highest CO\textsubscript{2} treatments were comparable to our results for control (and treatments) and to other *P. onkodes* collected from the Great Barrier Reef (Nash et al., 2011; Diaz-Pulido et al., 2014). This raises the possibility that CCA Mg-C levels are susceptible to change as CO\textsubscript{2} rises but only for levels higher than a stable baseline, which for the tropical corallines may be in the range of \(\sim 16–17.5\) mol\% MgCO\textsubscript{3}. Egilsdottir et al. (2012) working on the temperate articulated coralline *Corallina elongata* reported a significant decrease in Mg content for new structures formed under CO\textsubscript{2} 550–1000 µatm. For tips, branches and basal parts formed under the enriched CO\textsubscript{2}, Mg content ranged from 14.7–15.9 mol\% MgCO\textsubscript{3} and was not significantly different from controls (15.7, 15.2, 15.4 mol\% MgCO\textsubscript{3} respectively). On the other hand, structures growing off the base exhibited 16 \% MgCO\textsubscript{3} under control conditions but reduced in the tips, branches and basal plates of these new structures (15.1, 14.9, 15.3 mol\% MgCO\textsubscript{3}) at 550 µatm pCO\textsubscript{2}. These results suggest there is a different calcification process for the new structures compared to the tips, branches and basal parts and that this calcification process is sensitive to CO\textsubscript{2} but only up to 550 µatm.

Work on CO\textsubscript{2} influences on coralline algae crust-structure has to date been on temperate corallines (e.g. Egilsdottir et al., 2012; Ragazzola et al., 2012, 2013; Hofmann et al., 2011). Experiments on living tropical CCA calcification have focused on weight changes (e.g. Anthony et al., 2008; Comeau et al., 2013; Johnson et al., 2014) and impacts on existing crust mineralogy (Diaz-Pulido et al., 2014). There is little specific information known about calcification processes in tropical crustose corallines. However as this study and previous studies on mineralogy (Nash et al., 2011, 2013b; Diaz-Pulido et al., 2014) show, carbonates in CCA are not only Mg-calcite but can also include dolomite, magnesite and aragonite. It is clear that the net mass of CCA is a result of multiple mineral-forming processes. While all form within the biological structure it seems unlikely that infill dolomite, magnesite and aragonite are all the result of organism controlled calcification processes and instead are biologically induced. Thus experimental net weight changes for *P. onkodes* may not always be a reflection of changes for only Mg-calcite calcification and/or dissolution.
Aragonite can form as a result of parasitic endolithic bacterial activity within the CCA (Diaz-Pulido et al., 2014) and contribute to measured weight gain. In the Diaz-Pulido et al. study (2014) weight change was due in part to a mix of bacterial-driven carbonate destruction processes and abiotic aragonite precipitation as a result of calcium mobilisation by the endolithic bacteria. In the Johnson et al. (2014) study weight gain by CCA from locations downstream of the reef front was interpreted as indicating acclimatisation. However if there were more endolithic bacteria present in their downstream CCA than the reef front CCA, it is possible that the experimental fluctuating conditions with elevated CO$_2$ activated bacterial processes and the lower CO$_2$ resulted in increased re-precipitation of mobilised calcium as aragonite (aragonite re-precipitation transforms the porous crust to dense cement) which could account for a proportion of the weight gain. Therefore it is problematic to presume acclimatisation based on weight gain without first checking how the weight was gained. The published experiments referred to in this discussion were all conducted prior to the discovery of the extra minerals in tropical CCA, but future studies should consider the more complex nature of mineral composition of _P. onkodes_ when attempting to explain weight changes and calcification (e.g. Nash et al., 2013a).

The varied responses of the tropical and temperate corallines to altered CO$_2$ indicate that the uptake of Mg by corallines is not consistent across all species or even within the same organism (Egilsdottir et al., 2012). Furthermore, the use of different methods of measuring magnesium concentration potentially complicates comparisons across data sets. Ries (2011) and our study used XRD to determine mol% MgCO$_3$. This measurement only returns mol% for the Mg-Calcite component and is not influenced by the presence of magnesium in other forms, e.g. dolomite or within organics, or diluted by the presence of aragonite. Kamenos et al. (2013) used Raman spectroscopy for identifying mol% MgCO$_3$ changes, this method is not widely used for coralline algae mineralogy studies. Egilsdottir et al. (2012) used inductively coupled plasma-atomic emission spectroscopy (ICP-AES) to quantify bulk magnesium and Ragazzola et al. (2013) used electron microprobe to obtain an average elemental composition for Mg/Ca ratios.
These methods return bulk magnesium for the total sample or portion under the electron beam and may be skewed by undetected aragonite, common in corallines (Smith et al., 2012; Nash et al., 2013b) or presence of Mg not within the Mg-calcite, (e.g. Caragnano et al., 2014).

4.3 Dolomite formation within 12 weeks

Prior to the discovery of bio-mediated dolomite in association with bacteria (Vasconcelos and Mackenzie, 1997) and CCA (Nash et al., 2011,) dolomite was thought to form by chemical alteration of limestone over geological time frames, e.g. thousands to millions of years (e.g. Saller, 1984). Although it has also been controversially argued that dolomite was the primary precipitation in some ancient dolomite formations (Tucker, 1982). Our experimental results demonstrate that bio-mineralised dolomite formation is rapid and occurring contemporaneously with the surrounding limestone formation. The apparent reduction in dolomite formation in the experimental conditions compared to the pre-experimental growth indicates that there is also a rapid response to changing environmental conditions. Accordingly, any interpretation of past environments made using dolomite that may have had a biological origin, i.e. dolomite in formerly shallow tropical environments, would need to take into account this potentially rapid formation and response to environmental change.

4.4 Implications for interpreting the geological past

The absence of a significant effect of CO$_2$ on dolomite formation in this experiment suggests that the observed correlation in the geologic rock record of dolomite and greenhouse conditions may not be a direct result of high CO$_2$ driving increased primary bio-mineralised dolomite formation. However, as noted in previous work (Nash et al., 2013a; Diaz-Pulido et al., 2014) dolomite is more resistant to chemical dissolution and biological erosion than Mg-calcite (and presumably also calcite). Therefore the positive correlation of dolomite and greenhouse epochs in the rock record (e.g. MacKenzie...
et al., 2008; Wilkinson and Given, 1986) may be due in part to preferential preservation of bio-mineralised dolomite compared to surrounding skeletal material, rather than CO$_2$ or temperature driven biological processes leading to increased dolomite formation. Furthermore, during greenhouse times, sea level was higher thereby providing greater area of warm shallow (epeiric) seas and thus more accommodation space for calcifying algae that may have formed dolomite. While past primary bio-mineralised dolomite levels may not have been directly linked to CO$_2$ levels, there is certainly support from other work (Nash et al., 2013a; Diaz-Pulido et al., 2014) for indirect biologically-associated processes leading to increased abundance of bio-mineralised dolomite under higher CO$_2$ conditions.

4.5 Changes in calcification in experimental tanks

Considering the aragonite observed in the crust where the CCA was transferred to the experimental tanks, it may be that interruptions to normal growth after transfer to experimental tanks, allowed seawater to penetrate into the shallow surface layer resulting in alteration of Mg-C to aragonite. Previous experiments on calcification rates of CCA found that rates of photosynthesis, and production of inorganic and organic carbon, were significantly lower in experimental tanks than in situ (Chisholm, 2003). A decrease in photosynthesis and calcification rates may be the explanation for the observed differences in calcified crust in this study, although the exact mechanism leading to the change is not known. The absence of the organic film in the experimental growth (Fig. 5c) raises the possibility that it is the absence of these organics that has led to the observed differences in calcification. Reduced organic production may also lead to less dolomite as experiments have shown that dolomite nucleates on polysaccharides produced by red algae (Zhang et al., 2012). It is probable that our experimental results underestimate how much dolomite could be formed in the open marine environment over a 3 and 6 month period.

The observation that the change to experimental tanks coincided with changes in CCA calcification has implications for extrapolating experimental results back to the
natural environment. There is a substantial change in the ultra structure and secondary mineralisation (i.e. formation of dolomite) processes. While comparisons between treatments are reliable, exact rates of calcification for *P. onkodes* are likely to be understated in experimental conditions compared to the open reef. This is an area that requires further work to determine what is causing this difference in calcification and if it is common to all similar experiments. Flow and wave energy will be important factors that influence the calcification processes and should also be considered in future aquarium designs that seek to test the effects of future acidification scenarios on CCA’s.

4.6 What does Mol% Mg-Calcite means for the CCA physiology and reef processes in a changing climate?

There have been no studies to date which explore the drivers of organism controlled calcification in the key reef-builder *P. onkodes* and what role the Mg content plays in this. Thus it is unclear at this time what influence the mol % MgCO$_3$ has on CCA physiology and reef processes and even more difficult to anticipate what may happen in the future in a changing climate. Early studies on Mg-C CCA dissolution rates (Plummer and Mackenzie, 1974; Bischoff et al., 1987) used CCA that had dolomite and possibly magnesite (see Nash et al., 2013a for discussion) therefore those results were a mix of dissolution rates for 2–3 different magnesium minerals, not just for Mg-calcite with different phases of mol % MgCO$_3$ as was interpreted. Much of our present understanding of biogenic Mg-C dissolution is based on those interpretations (e.g. Andersson et al., 2008). Considering how recent work on CCA dissolution has revealed that a complex suite of interacting mineral, biological, bacterial and chemical factors contribute to net dissolution responses (Nash et al., 2013a; Reyes-Nivia et al., 2014; Diaz-Pulido et al., 2014) it has become apparent that the prevailing theory that higher Mg content leads to lower stability is probably not applicable to tropical CCA *P. onkodes*. Indeed there have been no dissolution experiments comparing the dissolution rates of CCA with different mol % MgCO$_3$ to test the correlation of dissolution rates to magnesium content of Mg-C.
4.7 Implications for reef management

Finding that dolomite is not affected by ocean acidification in these 3 and 6 month experiments is good news for the survival of CCA species *P. onkodes* under predicted ocean acidification conditions. Dolomite confers stability on the CCA and facilitates its reef-building role (Nash et al., 2013a) as well as being resistant to bacterial bio-erosion (Diaz-Pulido et al., 2014). At this time exact drivers of CCA dolomite formation have not been identified. It seems most likely that dolomite formation is related to provision of a suitable organic substrate, probably being the polysaccharides derived from red algae for agar (Nash et al., 2013a; Zhang et al., 2012). For coral reef management, it is necessary to understand what environmental conditions negatively impact dolomite formation. CCA crust formation is likely to suffer negative effects from reduced recruitment, increased bleaching, bio-erosion and dissolution under higher CO$_2$ and temperatures (Kuffner et al., 2011; Diaz-Pulido et al., 2012). However, understanding the conditions which negatively impact dolomite formation may enable more effective assessments of the risk that CO$_2$-driven ocean acidification may pose to important reef-builders such as *P. onkodes*. Identifying the drivers and constraints of CCA dolomite formation is an area of research that has not yet been initiated and as such, there is a long way to go to understand what conditions may negatively impact on CCA dolomite formation.

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References


Ocean acidification does not affect magnesium composition or dolomite formation

M. C. Nash et al.

Tucker, M. E.: Precambrian dolomites: petrographic and isotopic evidence that they differ from Phanerozoic dolomites, Geology, 10, 7–12, 1982.


Table 1. Two factor analysis of variance (ANOVA) testing for difference in mol\% MgCO$_3$ and Asymmetry indicating dolomite, between different CO$_2$ treatments (factor treatment) and experimental growth vs. pre-experimental growth (factor type). No significant difference related to CO$_2$ treatments, but significant difference between experimental and pre-experimental growth for both mol\% MgCO$_3$ and dolomite asymmetry.

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Figure 1. Example of *Porolithon onkodes* (CCA) after 3 months. New pigmented crust over-growing resin used for XRD.
Figure 2. Magnesium composition for experimental growth of P. onkodes. Mol % is for Mg-calcite mol % MgCO$_3$. Asymm mol % includes influence of dolomite asymmetry on calculated Mg-calcite mol % MgCO$_3$, the more dolomite present the higher the Asymm mol %. (a) New crust after 3 months. (b) New crust after 6 months. There was no significant difference between treatments for either the mol % MgCO$_3$ or the Asymm mol % in new crust after 3 or 6 months. * control samples were unavailable for mineral analyses. (c) New settlement after 6 months. As for the new crust, there was no significant difference across the treatments in mol % MgCO$_3$. There is no dolomite in the new settlement consistent with the absence of white crust. Error bars are ±1 SD.
Figure 3. Magnesium composition for CCA new settlement, 3 month crust, 6 month crust, and pre-experimental crust. The mol% MgCO$_3$ in the Mg-calcite increases from new settlement to 3 and 6 months, and again for the pre-experimental crust. Dolomite is not present in the new settlement, appears within 3 months, increases in amount in the 6 month new crust, but is highest in the pre-experimental crust. Error bars are 1 SD.
Figure 4. SEM of control *P. onkodes* showing dolomite in experimental and old growth. (a) Experimental growth–dolomite (D) Dolomite-composition material in cell. This is not the typical cell lining but has been observed in other CCA. Mg-calcite (Mg-C). Scale = 2 microns. (b) Experimental growth: micro-scale lumpy dolomite lining cell. Unknown if this is the precursor to the typical cell lining observed throughout the main crust and other CCA (e.g. Nash et al 2011). Scale = 1 micron. Cell growth in experimental growth is less regular and organized than old growth. (c) Dolomite cell lining in old growth. (d) Dolomite infill in a reproductive conceptacle in the old growth. Cells below conceptacle are all in-filled with dolomite. Scale bars: A and C = 2 microns, B = 1 micron, D = 10 microns.
Figure 5. Control *P. onkodes* with experimental growth on pre-experimental growth. (a) There is a visible difference in the appearance of experimental crust (black arrow) to the old growth (black dashed arrow). The lighter grey of the surface is due to less magnesium (dolomite) infilling the cells that appear as darker grey infill in the pre-experimental lower part of the crust. Black box enlarged in B-D is dolomitised conceptacle. (b) Close up showing the consistent presence of infill in pre-experimental growth whereas the new growth regular dolomite cell lining is absent. Also, the Mg-C crust itself appears to be less dense with many cracks from the cutting visible in the new growth but not so in the pre-experimental growth. (c) Secondary electron image of control CCA. The pre-experimental growth appears to have a fine opaque organic film covering part of the cut crust (white dashed arrow), but this is not present in the experimental growth (white arrow). (d) Control CCA (BSE) Dashed arrow to pre-experimental growth. Grey cells are dolomite infill. Black arrow to experimental growth, generally an absence of dolomite infill, note line of porosity in transition between pre-experimental and experimental growth. Scale bars: A, C and D = 100 microns, B = 20 microns.
Figure 6. Transition from pre-existing crust to experimental crust in *P. onkodes*, pre-industrial CCA (a, b), CO$_2$ CCA (c, d). (a) Overview, brackets- new growth. (b) Close up of transition. Crust below dashed line is pre-experimental growth. Dolomite infills cells (black arrows). Above dashed line new growth does not have cells infilled, crust has been damaged by saw cut. (c) Overview of transition to new growth in high CO$_2$ CCA, brackets – new growth. (d) Close up of transition. Similarly to control and pre-industrial CCA, cells in pre-experimental growth are infilled with dolomite (black arrows). Crust above dashed line grew during experiment. Cells are not infilled with dolomite and crust has crushed under the sawcut. Scale bars A, B, C and D = 20 microns.
Figure 7. SEM of Control *P. onkodes* (AC4). (a) Overview of experimental growth, pre-experimental growth and transition zone (bracket). Cells at the surface do not have dolomite and this matches with XRD results of pink growth layer and observations in other work (Nash et al. 2011, Diaz-Pulido et al. 2014). White box enlarged in B. (b) Cells in experimental growth have no dolomite infill. Cells below experimental growth have dolomite lining the cells but the centres are in-filled with aragonite. White box enlarged in C, black box enlarged in E. (c) Close up of cell infill by aragonite within the dolomite lining. (d) Dolomite lined cell in transition zone with aragonite infill. (e) Patch of crust below experimental growth with aragonite infill. (f) Close up of dolomite-lined cell with aragonite infill. Scale bars: A and B = 20 microns, C and F = 1 micron, D = 2 microns, E = 10 microns.
Figure 8. Varied mineral fabrics in CCA. (a) Alteration of base of CCA crust by bacteria to aragonite (Diaz-Pulido et al., 2014), remnant CCA cells are visible in the aragonite confirming it was CCA crust and not coral substrate. (b) Hypothallus cells grow parallel to substrate then grow vertically and are in-filled with dolomite. In-fill of micro-borer trace by aragonite and dolomite rim (arrow). (c) Band of dolomite between aragonite alteration and undamaged cells. (d) Damaged crust has been in-filled with new cell growth rich in dolomite. Scale bars: A = 100 microns, B, C and D = 20 microns.