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Interactive comment on "Ocean acidification does not affect magnesium composition or dolomite formation in living crustose coralline algae, *Porolithon onkodes* in an experimental system" *by* M. C. Nash et al.

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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 doesnt have a scale) or on an adjacent drawing.

Response-A paragraph defining the terminology used and linking to standard coralline terminology has been added after the methods. Extra references added 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 re-growth 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. "

Steneck, R. S. (1986). The ecology of coralline algal crusts: convergent patterns and adaptative strategies. Annual Review of Ecology and Systematics, 273-303.

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 looses 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?).

Response-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 buildup of epithallus growth after it looses its pigmentation.”

Adey, W. H., Halfar, J., & Williams, B. (2013). The coralline genus Clathromorphum Foslie emend. Adey: Biological, physiological, and ecological factors controlling carbonate production in an Arctic-Subarctic climate archive.

Pueschel, C. M., Judson, B. L., & Wegeberg, S. (2005). Decalcification during epithallial cell turnover in Jania adhaerens (Corallinales, Rhodophyta).Phycologia, 44(2), 156-162.

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?).

Response-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

Response-Edits made

Reviewer 2 Some legends have inferences in them move to discussion sections.

Response-Removed

P1384 L 24: could this be post depositional change?

Response- 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% MgCO3 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% MgCO3 (Nash et al. 2013b) suggesting if there is post-deposition Mg enrichment, it occurs relatively contemporaneously with growth."

Adey, W. H., Halfar, J., & Williams, B. (2013). The coralline genus Clathromorphum Foslie emend. Adey: Biological, physiological, and ecological factors controlling carbonate production in an Arctic-Subarctic climate archive.

Darrenougue, N., De Deckker, P., Eggins, S., & Payri, C. (2014). Sea-surface temperature reconstruction from trace elements variations of tropical coralline red algae. Quaternary Science Reviews, 93, 34-46.

Halfar, J., Adey, W. H., Kronz, A., Hetzinger, S., Edinger, E., & Fitzhugh, W. W. (2013). Arctic sea-ice decline archived by multicentury annual-resolution record from crustose coralline algal proxy. Proceedings of the National Academy of Sciences, 110(49), 19737-19741.

Kamenos, N. A., Cusack, M., & Moore, P. G. (2008). Coralline algae are global palaeothermometers with bi-weekly resolution. Geochimica et Cosmochimica Acta, 72(3), 771-779.

Fietzke, Jan, Federica Ragazzola, Jochen Halfar, Heiner Dietze, Laura C. Foster, Thor Henrik Hansteen, Anton Eisenhauer, and Robert S. Steneck. "Century-scale trends and seasonality in pH and temperature for shallow zones of the Bering Sea." Proceedings of the National Academy of Sciences 112, no. 10 (2015): 2960-2965.

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

Response- Reference to the results for the living, relevant to this experiment, has been added.

. . ..”Lithothamnion glaciale showed no changed 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 re 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).

Response- 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.

Response_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 insitu 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.

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”

Response- changed.

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

Response- changed

Pg 1375 L20: Rhodoliths

Response- changed

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

Response- changes made

Pg 1378 Section 2.3. First sentence is quite long, could be made into two smaller ones.

Response- 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.

Response- checked and changed throughout MS

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

Response- changed

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

Response- changed

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

Response- changed

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

Response- changed

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

Response-changed

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 $p < 0.05$).

Response- 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

Response – 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?

Response- 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?

Response-Yes-there are images similar to those in Fig in the supplementary information, Fig 3, referred to at line 24, page 1383. - Extra note added to legend to Fig 6 to refer again to SI Fig. 3 - 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.

Response- definitions added and edits made

Interactive comment on Biogeosciences Discuss., 12, 1373, 2015.