

Nash and Adey
Response to reviewers

We thank both the reviewers for their positive comments and suggestions. We appreciate their recognition for the importance of this work.

The technical edits have been made, excluding the suggestion to reorder the mention of figure 5 in the MS. This is mentioned before figure 2 as it is relevant to the methods section, however it is logically placed in the main text as figure 5 after the information presented in figures 1-4.

The editorial suggestions have been incorporated or addressed as detailed below.

Reviewer- A. Caragnano

L75-78 *add extra references, including Cabioch and Giraud.* Extra references added. Additionally, Cabioch and Giraud also now referred to in the discussion.

Suggestion to change epithallium to epithallum.

While we appreciate the lesson in Greek and latin, the latter which I certainly could have benefited from during my formal education, using the spelling with ium is common in current phycology literature and we do not propose to use spelling contrary to that currently applied in published literature.

L265-267 *long cut-* noted. The perithallial cells subject for the main comparison were similarly orientated. The hypothallial cells here were of secondary interest as their Mg was not measured, nor was there a substantial bulk of the crust as hypothallial growth. No changes made.

Line 288- Cabioch and Giraud were referring to the difference between the epithallial cells (not analysed in this study) and the perithallial cells. Thus, this is unlikely to be the explanation for the measured difference in this paper. In the discussion the following has been added to discuss the reason for the lower Mg:

The *K. epilaeve* perithallial cells had lower Mg than the *L. leave* perithallial cells.

Cabioch and Giraud (1986) described the *perithallial* cells as being a later stage of development than *epithallial* cells. Epithallial cells do not have fully developed rounded cell walls of the perithallial cells (Adey 2015a, b). Although Mg-content of epithallial carbonate is lower than the perithallial values (Diaz-Pulido et al. 2014, Nash et al. 2015, 2016), the lower Mg measured here is not considered a result of different cell type as the

K. epilaeve cell walls have the radial calcite similarly to the perithallial *L. Leave*, indicating that these are similarly well developed. Considering the time of collection in early summer, it is quite possible that the *K. epilaeve* growth closest to the *L. leave* surface was laid down closer to winter and in cooler temperatures, this being a likely explanation for the lower Mg content.

L404-

The reviewer suggests the affirmation that the Mg change is not related to temperature is not well supported.

We disagree. We have provide statistical support for our claim that the Mg is related to anatomy with the eds measurements for the cell wall v interfilament v hypothallial, all being statistically significantly different. In the MS we provide numerical comparisons for the difference in temperature that would be required to drive the Mg content change, temperature changes that are unrealistic for that geographical area. Since first writing this MS, further research we have undertaken has revealed the same Mg content patterns with anatomy for another general, *Phymatolithon*. This work has just been accepted for publication and we have added this reference (Nash and Adey 2017). We consider this extra data showing the same phenomenon supports our affirmation.

Reviewer J. Fietzke

Regarding the reviewers concerns that we suggest existing calibrations not to work. This is not our intention, nor do we believe they do not work. The information in this MS will aid to refine future calibration. However, understanding that a switch to elongated thin-walled cells can involve an anatomically –driven increase in Mg has relevance for temperature calibrations made using rhodoliths that regularly switch to elongate, thin-walled higher Mg cells. We have made the following 2 edits:

Edit 1 line 506

We do not suggest current studies are inadequate because the finer scale (submicron) scale variations are not captured. These fine scale variations will not change the general trends or conclusions. Rather, we suggest caution regarding interpretation of data where a change in Mg is visibly associated with a change in cell type as temperature may not be the only possible driver of Mg change.

Edit 2 line 542

The rhodolith summer cells have similarities in appearance to the hypothallial cells in this study. Possibly the higher measured Mg in the long cells of the rhodolith is a result in part of a switch towards a more perithallial style cell and may not be entirely temperature related. This proposition is supported by Sletten et al. (2017) who found a switch to elongated cells with higher Mg that was unrelated to seasonality.

More detailed quantitative evaluation of to what degree the seasonal variation in Mg content can be explained by changing skeletal structure.

To do the seasonal quantitative evaluation we would need samples with the seasons constrained experimentally by stain or other geochemical tracer. It would be very interesting to do this work and would help to understand exactly how much the changing proportion can influence the total values. Unfortunately it is not something that is planned at this time.

Or how much of the Mg variation is truly coming from a changed chemical composition of the calcite crystals formed and how much is reflecting changing skeletal structure.

The reviewer is correct in that this question has been the focus of later work. Some of this work showing the consistent change with temperature for both interfilament and cell wall has just been accepted for publication and the reference has been added. We have added a brief mention in the concluding discussion on the extra work and edited the paragraph appropriately

Edit Line 568

Recent work indicates that the interfilament and perithallial carbonate react similarly to temperature, but the responsive hypothallial carbonate is inconclusive (Nash and Adey 2017). It would be interesting to identify if each of interfilament, perithallial and hypothallial cell walls reacted similarly to changes in seawater Mg:Ca, or if there were differences in anatomical controls.

Reconsider the very short conclusion

We deliberately kept this short as it is the first of several papers investigating this topic and they each are getting longer and longer. We have however added a second short paragraph.

Edit

While the focus of this study has been the distribution of Mg with different anatomical features, the high-magnification images are the first to show the cellular-scale organic structures together with the carbonate components. The orientation of the crystals in the interfilament and the cell walls are in agreement with lower-magnification SEM studies on a range of algal species (Cabiocch and Giraud 1986, Adey et al. 2013). The

combination of gentle etching and high-magnification SEM has revealed previously unknown features such as the fibrils threading through the radial Mg-calcite (Fig. 7C). Further, showing that the Mg content varies with anatomical features suggests that the calcification may be a different process, or have different controls, for each carbonate type. This adds an extra level of complexity when considering how environmental changes, such as increasing temperature, may impact on the capacity of the CCA to continue their important substrate provision ecological role.