

Biogeosciences Discuss., author comment AC1 https://doi.org/10.5194/bg-2021-21-AC1, 2021 © Author(s) 2021. This work is distributed under the Creative Commons Attribution 4.0 License.

Reply to Referee#1

Giulia Piazza et al.

Author comment on "Growth rate rather than temperature affects the B/Ca ratio in the calcareous red alga *Lithothamnion corallioides*" by Giulia Piazza et al., Biogeosciences Discuss., https://doi.org/10.5194/bg-2021-21-AC1, 2021

Dear Referee #1,

Thank you for your accurate review and for the time you spent in carefully reading our work. Our paper has benefited a lot from your suggestions. We are also glad for your appreciation on the scope of our research and that you recognize the uniqueness of our data.

Hereafter I will reply to each of your comments.

- **Comment:** The authors say in the abstract "We produced the first data on temperature proxies (Mg, Li and Sr/Ca)" but it would be really nice to see more made of these important data. If these results are to be used to establish temperature dependency (or not) on B/Ca, the temperature dependency of Mq Li and Sr must first be convincingly made first. Many papers are cited here that show the correlation between these variables on seasonal cycles, but it has not been established that temperature is the main driver of the incorporation over seasonal growth rate. The use of Li/Ca in particular as a "temperature proxy" requires more consideration. The Darrenougue paper cited does indeed shows that Li/Ca is high in the warm summer months, but it is not clear that this is driven by temperature. Li has a very low partition coefficient, so as growth rates increase during summer kinetics and growth entrapment models would suggest that Li/Ca go up. This growth rate dependency may mask the Li/Ca temperature effect which some have argued is a negative slope in carbonates and should be ratioed to Mg for reliable temperatures (Anagnostou et al., 2019; Stewart et al., 2020 EPSL; Marriott et al., 2004 EPSL and Chem Geol.). It would be interesting to see where these Li/Mg data fall on calibration lines of Anagnostou et al., 2019 and Stewart et al., 2020 (EPSL). Do they agree with the linear fit of Anagnostou for cultured CCA or the speculative exponential relationship found across all high-Mg calcites suggested in Stewart et al., 2020? The Li/Mg results here (I am estimating as raw Li/Ca ratios were not provided in a table) seem perhaps a little high compared to Anagnostou culture data, but this could be due to analytical offsets that can occur during laser ablation analysis compared to solution chemistry.

Response: We agree with you that it would be extremely interesting to quantify the temperature and growth rate dependency on Mg, Li and Sr. We also agree that the application of the Li/Ca proxy is still controversial, whereas Mg/Ca has a widely recognized reliability as temperature proxy in coralline algae. Nevertheless, Li/Ca has been proven to be a reliable seawater temperature proxy in coralline algae (Caragnano et al., 2014; 2017, Anagnostou et al., 2019), and a Mg/Li calibration did not improve the Mg/Ca or Li/Ca temperature relationship in previous field studies (Caragnano et al., 2014; 2017). We

have plotted our Mg/Li data on the calibration line of Anagnostou et al. (2019), as you suggested (new Figure 6). As you can see, our data fall in the range of values found by those authors. Nevertheless, our Mg/Li results did not show significant correlation with temperature. This has been evidenced also by the lack of seasonal oscillations in Mg/Li values found in Morlaix (new Figure 7). Therefore, Mg/Li is not considered a significant temperature proxy for the purpose of this work. These results have been added to the revised paper. In our paper, Li/Ca data (as well as Sr/Ca) have a significant positive correlation with Mg/Ca, which is considered mainly controlled by temperature. Therefore, we confirm other studies which suggest a dominant control of temperature also in the Li incorporation. Anagnastou et al. (2019), which you cited, performed geochemical analyses on the cultured coralline algal species *Clathromorphum compactum* and found an inverse relationship between Li/Ca and seawater temperature. We recognize the importance of culture studies to understand the geochemical equilibrium between these calcifying organisms and seawater chemistry. Nevertheless, data collected in the field on wild-grown specimens, do not allow us to perform such high-resolution calibrations, that were beyond our research purpose. This point will be further discussed in the next responses.

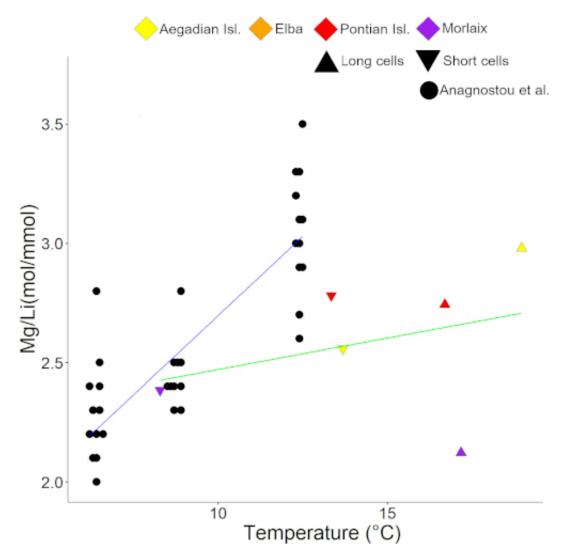


Figure 6 (new): Correlation plot between Mg/Li and seawater temperature. Data are shown for cultured *C. compactum* (Anagnostou et al., 2019) and *L. corallioides* (this paper). *L. corallioides* results are shown separately in long and short cells, per sampling site.

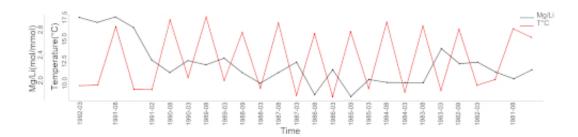


Figure 7 (new): Mg/Li ratio of *L. corallioides* collected in Morlaix Bay. Note the lack of cyclic variations in Mg/Li results. In the timeline, the coldest and the warmest months have been reported. Mg/Li in the missing bands have been calculated as the means of the values measured in warm and cold periods. Monthly means of seawater temperature have been extracted by ORAS5 reanalysis.

- **Comment:** Do the short or long cells measured offer a better temperature reconstruction? It would be great to see the authors bring this into the discussion and would add to the interpretation of these elemental ratios as being temperature dependent thus permitting their use to unpack competing effects on B/Ca.

Response: The separation of short and long cells definitely allowed to showcase the correlation of skeletal pattern and Mg incorporation. We agree that there are multiple factors influencing the element/Ca in coralline algae, as well as in other calcifying organisms. Nevertheless, to measure the influence of each single factor, laboratory experiments would be needed. In our paper, the distinction between the geochemical signal from short and long cells allowed us to evaluate the banding variations in the element content. This was useful to investigate, for the first time, the B/Ca variability across the algal thallus. Other considerations are object of ongoing investigation and beyond the aim of this contribution.

- **Comment**: Of particular concern, the main finding that growth rate is driving B/Ca is based on a correlation of just 4 data points (figure 9), three of which have very similar growth rates. The lack of variety of environmental conditions and growth rates means that, despite the presentation of p values, the significance of this relationship is highly questionable. I cannot see that the authors are in a position to establish the outright controls on B/Ca with the data collected. The 4 samples presented here, 3 of which are in the Med, simply do not cover a wide enough array of hydrographic conditions to pick these effects apart.

Response: We believe that the strength of our paper relies on the fact that it is the first work comparing the same coralline algal species across a natural gradient, across different depths and even different Basins. This implies both strengths and limits, since it allowed us to observe the response of a single widely distributed species in natural environmental conditions, but with a limited set of variations compared to culture experiments in the laboratory-controlled conditions. Moreover, we would like to note that one of the main differences in working with wild-grown specimens is the impossibility to control and check for each environmental factor influencing the calcification during growth. As you highlighted above, there are multiple factors influencing the geochemistry of these organisms and the best that we can do is to collect as much data as possible to take the big picture, then identifying the main contributing factors to the variations we observe. No coralline algae live in any possible environment, and actually our "4 points" derive from several observations and several samples collected in the natural environment. Please note that the Mediterranean is not an experimental tank in controlled conditions, because it shows a wide range of possible combination of environmental controls. In other words, the pattern shown in Fig. 9 (new Figure 11) has an intrinsically strong significance for L. corallioides.

- **Comment:** Given the uncertainty on driving factors of all of these elements, not just B, I would recommend the authors take a more explorative approach to their results rather than categorically stating which are temperature dependent. It would be useful to see all elemental data plotted as a time series to see if there are trends along the growth axis and the cyclical nature of each elemental ratio with season. This could be compared to the CA image to show the matching cycles to light and dark banding. It would be great to see these annual banded timeseries matched to gridded SST records for each site (e.g. ERSST https://www.ncdc.noaa.gov/data-access/marineocean-data/extended-reconstructed-seasurface-temperature-ersst-v5 or HADISST

https://www.metoffice.gov.uk/hadobs/hadisst/data/download.html). This would really help to convince the reader about the temperature control on each element. In short, I would very much like to see these important data published, but with a more measured approach to the discussion.

Response: We agree on the complexity of interpreting the driving factors of element/Ca and the text has been modified for less straight forward conclusions. We also agree that the paper could benefit from adding a plot showing the time series of all elemental data, highlighting the elemental cyclicity, as you suggested. We have therefore provided the new plot and we thank you for your suggestion (new Figure 12).

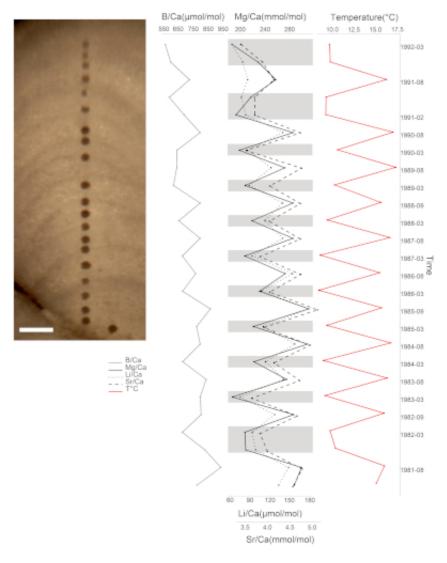


Figure 12 (new): Elements ratio of *L. corallioides* collected in Morlaix Bay (scale bar = 200 μ m). Mg, Li and Sr/Ca show cyclic variations the same as the local seawater temperature. In the timeline, the coldest and the warmest months have been reported, which

correspond to dark and light bands of growth. Elements/Ca in the missing bands have been calculated as the means of the values measured in warm or cold periods. Monthly means of temperature have been extracted by ORAS5 reanalysis.

- **Comment:** Line 16: "from shallow to deep waters" seems misleading. None of these are truly deep waters. Perhaps "from across the photic zone depths" would be better.

Response: The text has been modified.

- Comment: Line 22: "This evidence suggests"

Response: The text has been modified.

- **Comment:** Line 32: pKB is pressure, temperature, and salinity dependent therefore the value of 8.6 is only applicable to surface waters (c.f. the value in deep waters is 8.8). Be clear that this is not a constant and that this refers to "typical surface seawater conditions"

Response: This has been specified in the text as suggested.

- **Comment:** Line 35: The Klochko value for the fractionation factor is more commonly given in the 1.0272 format.

Response: The text has been modified.

- **Comment:** Line 35: "Seawater isotopic composition $\delta^{11}B_{sw}$ is 39.61‰ (Foster et al., 2010) and varies with the isotopic composition of $B(OH)_3$ and $B(OH)_4^-$, both enriched in $\delta^{11}B$ with increasing pH (Dickson, 1990)." This sentence is poorly worded. Seawater $\delta^{11}B$ does not vary with the isotopic composition of boric acid and borate. Rather the $\delta^{11}B$ of seawater is fixed and the 11/10 ratios of boric acid and borate have to change to maintain the fractionation factor as their abundances change with changing seawater pH.

Response: The text could be misunderstanding and has been modified for clarity as you suggested.

- **Comment:** Line 50: It is worth stating that these $B/Ca vs CO_3^{2-}$ relationships are only empirically derived observations.

Response: This has been specified as suggested.

- **Comment:** Line 52: Paragraph starting "Warming and acidification..." should be the opening of the introduction leading to a statement about proxies for the carbonate system are therefore much needed. The discussion of $\delta^{11}B$ and B/Ca (currently starting at line 25) will then follow better.

Response: This change has been made.

- **Comment:** Line 55: Unclear what is meant by "longevity by indeterminate growth,". As outlined in the next sentence many specimens have annual growth bands therefore we can determine their growth axis and age.

Response: The "indeterminate growth" is a botanical concept that has been widely referred to coralline algae (Adey, 1965; Frantz et al., 2005; Halfar et al., 2008), and it is held responsible of their longevity. It contrasts with the growth pattern of bivalves, for example, which are influenced by an ontogenetic growth trend which slows down with age following an asymptotic curve. This concept is very important for climate reconstructions

since an ontogenetic trend would compromise the resolution of the geochemical signals in the later stages of growth. Coralline algae, instead, do not slow growth over time and the element incorporation is not governed by age. We understand that this concept should be better explained for non-specialists and we added a more detailed explanation in the text along with the citation.

- Comment: Line 78: "culture experiment"

Response: The text has been changed.

- **Comment:** Line 80: Suggested rewording: "The factors which influence the B incorporation in CA are therefore still debated."

Response: The text has been changed.

- **Comment:** Line 88: I think Hetzinger et al., 2011 should be Hetzinger et al., 2009. The 2011 paper is about Ba rather than Mg Li or Sr

Response: Actually Hetzinger et al., 2011 shows Mg and Sr data besides Ba, while Hetzinger et al., 2009 is only about Mg/Ca.

- **Comment:** Table 1 and 2 could be combined, that way the reader can see how the collection depth compares to the Temperature data. Also strange to have table 2 A and B – all of these columns could be combined into one table. Please consider if all columns are necessary. For example, Lat and Long could be moved into the caption of Fig1. If you have Temp max and min, do you need to spell out the range? Do you even need the range when you have the st. dev? Is standard dev not a better measure of the variability at each site than the range and demonstrates the point about the Atlantic being more variable on its own? It is also strange to present this info in a table and then take up words spelling it all out again in the main text (section 3.1 can be very much shortened).

Response: Table 1 and 2 have been combined as you suggested. Concerning the temperature range, we believe it is important to explicit these data since they have a different meaning from standard deviation. ΔT has indeed been measured as the difference between maximum and minimum temperature values extracted in the sampling site during the considered time interval. These data better explain the different temperature variations experienced by the alga during its growth and are directly related to the sampling depth. We agree that section 3.1 could be shortened just citing the data in the table and changes have been made to the text accordingly.

- **Comment:** It would be nice to see all trace metal data tabulated somewhere too.

Response: A new table has been added summarizing the elemental data resulting from the work (Table 2). Moreover, raw data have been submitted to the PANGAEA repository (see Supplement).

- **Comment:** Table 2 caption. It is not clear what is meant by data "elaboration". Please consider wording here.

Response: The text has been modified for clarity.

- **Comment:** Line: 118: "Particularly,..." should read "In particular,..."

Response: The change has been made.

- Comment: Line 121: Suggest rewording "Once its inclusion under the genus

Phymatolithon was excluded, the Morlaix sample..."

Response: The text has been modified as you suggested.

- Comment: Line 133: Suggest "Element/Ca ratios were calculated for these isotopes,"

Response: The text has been modified.

- **Comment:** Line 133: What about these elemental ratios was "in agreement with Yu et al., (2005) and Darrenougue et al., (2014)"? Presumably Yu and Darrenougue didn't measure these same samples, but did they measure the same standards? Was it the methods that are similar? It is not clear what is mean here.

Response: Those authors measured the same isotopes in different samples. We have removed this sentence which could be misunderstood and does not add significant information to the methods.

- Comment: Fig 2: Please label long and short cells on panel b

Response: We have added the labels.

- **Comment:** Line 140: this method section requires more information. What is the measurement accuracy and precision? NIST 612 was used as an "external standard" (does this mean a bracketing standard?), but were any other reference materials used to demonstrate measurement accuracy (e.g. JCp-1 coral powder pressed as a pellet)? Bracketing with NIST glass for carbonate laser ablation is far from ideal in terms of matrix matching, but others have demonstrated that it can give reasonable results (Fietzke et al., 2010).

Response: We did not analyse other standards for quality control. Following your suggestions, we added the references of Fietzke et al. (2010) and also Jochum et al. (2012), which demonstrated the reliability of NIST glass standards.

- **Comment:** Line 150: Suggest: "Carbonate system parameters for each site have also been estimated"

Response: The text has been changed.

- **Comment:** Line 160: suggest: "Growth rates were estimated under light microscope by measuring..."

Response: The text has been changed.

- Comment: Section 2.5. Suggest simpler subtitle "Statistical analysis"

Response: The subtitle has been modified.

- **Comment:** Line 178: Lower amplitude seasonal temperature change. The word "excursion" implies an aberration from the norm, this doesn't fit when describing an annual cycle.

Response: The text has been corrected.

- Comment: Line 179: "site" rather than "one"

Response: The text has been changed.

- **Comment** : Line 189: The pH gradient described here doesn't really exist as described. pH estimates at the Mediterranean sites are all similarly high ~8.13 and less variable than the Atlantic site (~8.06). Similarly for DIC, as this is largely dictating the pH

Response: The results have been reported in the text following your suggestion.

- **Comment:** Fig 3: p values of 2.2e⁻⁶ could just be written as p<0.01 as the extra decimal places after the 99% offer little additional information to the reader. Also no need to repeat these R and p values in the main text if they are in the figure. Title of "spearman correlation" should be removed as figure titles are redundant with a caption and this info is already in the caption. It this all data or one CA sample? It would be interesting to see how points vary by location. Perhaps colour code the points or use symbols for each site (this could be applied throughout to all figures, including the map, for consistency). Change Y and X of regression lines to Mg/Ca and Li/Ca

Response: All your suggestions on Figure 3 have been followed and the plot has been changed accordingly.

- **Comment:** Section 3.2 more repetition of mean values that are shown in Figure 4. Consider making the Results section more concise throughout using the information presented in the figures and tables rather than repeating.

Response: The repetitions have been deleted throughout the text of the Results sections, improving readability. Thank you for your suggestion.

- **Comment:** Line 216: "areas" rather than "spots" and "within light growth bands" rather than "positioning on"

Response: The text has been changed.

- **Comment:** Box plot figures would make more sense if the sites were ordered Aeg, Elba, Pontian, and then Morlaix so that the Med sites are next to each other and the Atlantic site. Given the similarity of the Med sites hydrographic data effectively two environments are being compared: the Med and the Atlantic.

Response: Box plot figures have been modified as you suggested.

- **Comment:** Line 231: see mainpoint above. It is important to establish which direction Mg, Li, and Sr are expected to change as temperature changes. It remains unclear to what extent these elements themselves are driven by growth rate and to what extent they are driven by temperature. A full discussion of their partition coefficients to establish their respective response to growth rate is required before temperature effects can be established and used to unpack the effects on B/Ca.

Response: As already explained in the response of your main point above, the information collected directly from a natural environment arises interpretation challenges. Our approach was to test the proxies in natural conditions where we cannot monitor the parameters influencing the algal growth as we could do in a controlled experimental setting. Therefore, we inferred the dependence on growth rate and temperature using the data we possessed without the possibility to perform calibrations and the discussion you were referring to. We decided to take reasonable approximations. Mg/Ca is considered a confident temperature proxy in coralline algae and we have no reason to doubt its reliability, since our results also show cyclic variations on Li and Sr/Ca temperature dependence had followed accordingly. We do not have the evidence to assume a major control of growth rates rather than temperature on Mg, Li and Sr incorporation.

- **Comment:** Line 237: "shows" rather than "evidenced"

Response: The text has been changed.

- Comment: Line 251: Showed an analogous trend to what? Please be clear here

Response: The text has been modified in "showed lower values in the Mediterranean sites and higher values in the Atlantic site".

- **Comment:** Section 3.4 these extension rate results should be included in the combined table 1 and 2. It is noteworthy that the growth rate at Pontian was lower despite its similarities in other environmental conditions with the other Med sites.

Response: The growth rates have been added in the new combined Table (Table 1).

- **Comment:** Line 277 This begs the question how often did light and dark band match Mg/Ca patterns. A figure would be useful to show this matching to convince the reader that there is indeed a Mg/Ca pattern that fits the colour banding.

Response: The plot showing the cyclical variations of Mg/Ca and other elements across the algal thallus has been added as mentioned before (new Figure 12).

- **Comment:** Figure 9: As with all figures, please identify the sites by colour or data point.

Response: Figures have been modified according to your suggestions.

- **Comment:** Line 280: consider wording here. Mg/Ca ratios do not go "negative". Suggest high and low

Response: Positive/negative peaks have been changed to high/low as suggested.

- **Comment:** Line 287 to 295: It is not surprising that the ranges in B/Ca measured here using a laser (capable of picking up more heterogeneity within samples) are wider than the bulk carbonate analyses measure by solution ICPMS performed by Anagnostou and Donald. Sure, it might be environmental, but the analytical difference must be acknowledged.

Response: Thank you for your comment. The different analytical methods have been pointed out in the text while comparing the literature data.

- **Comment:** Line 299: I think this statement is rather unsupported. It has been established that Mg data covary with Li and Sr, not that Li and Sr are temperature dependent. This could be growth rate particularly given the statement on line 307 about temperature covarying with irradiance.. Also, again, the use of "deep water" is misplaced.

Response: As you pointed, we provided evidence that Mg data covary with Li and Sr. Nevertheless, since the major factor controlling Mg/Ca in coralline algae is temperature, this covariance suggests that temperature is also a determinant factor in controlling Li and Sr/Ca. Definitely, multiple factors contribute at the same time in the elements incorporation, including growth rates and light availability. In natural environments as in our study, we could not control all the factors influencing the algal growth and we interpreted the data we obtained without a constant monitoring. Moreover, the new plot showing the cyclical variations of elements/Ca through time, as you suggested to add, clearly shows a correlation between temperature and Mg, Li and Sr/Ca, contrary to B/Ca (new Figure 12).

- **Comment:** Line 319: This is also little consensus on the B/Ca effect in biogenic carbonates. New coral culture data by Gagnon et al., 2021 ESPL (see supplement) show that B/Ca increases with carbonate ion when DIC is held constant, and decreases with carbonate ion when pH is constant. This paper also shows a strong negative DIC effect on B/Ca (unlike the inorganic experiments of Uchikawa) however this may be strongly related to scleractinian biocalcification mechanisms and the rate of replenishment of the calcifying fluid with seawater.

Response: Thank you for the reference, which is very interesting. It will be cited in the text along with the negative correlation between [DIC] and B/Ca found by Donald et al. (2017) in a cultured coralline alga. This could contribute to the high B/Ca value found in Morlaix compared to the Mediterranean sites.

- **Comment:** Line 321: check the DIC value. I think this should read 2.32 rather than 8.23. Also this value extremely similar to all of the other Med sites and is therefore unlikely to be driving B/Ca. Indeed the lowest DIC site (Morlaix) also has a high B/Ca (>700). This is acknowledged later on, but the statement about DIC causing the high B/Ca at Elba is completely unsupported by the results at the other sites.

Response: The typing error has been corrected and the discussion has been changed to better support the results, as mentioned in the previous response.

- Comment: Line 333: "suggested" rather than "proved"

Response: The text has been changed.

- **Comment:** Line 343: Similar to my point about the questionable significance of a correlation between just 4 data points, the relationship between B/Ca and ΔT could be considered absent or strongly positively covarying if Elba is considered anomalous. There are just not enough data to say either way. It is also not entirely clear why the magnitude of the seasonal cycle (ΔT) should be correlated to B/Ca and temperature itself not? This needs further explanation.

Response: We decided to focus on ΔT , which was calculated as the difference between the maximum and minimum seawater temperature registered during the algal growth. The definition of ΔT and its meaning has been added in Materials and Methods section. As mentioned above, we believe that this measure better characterizes the sampling sites compared to absolute temperature, given their differences in depth and geographical regions. Elba and Pontian Isl., for example, have both a temperature mean of 15°C. Nevertheless, in Pontian Isl. (66 m depth), the temperature keeps more constant throughout the period ($\Delta T = 3^{\circ}C$), while in Elba there is a higher amplitude of temperature variations ($\Delta T = 5^{\circ}C$). The effects of these fluctuations around the optimum of algal growth would not have been highlighted by the mean temperature value. Last, but not least, the resolution of the laser ablation does not allow us to precisely discriminate the month of year the analysis is referring to. Therefore, we cannot attribute a single point of analysis to an absolute temperature in a specific time of the year, but rather refer more generically to the cold and warm season. In fact, dark and light bands are usually associated to cold and warm periods and we have used this estimation to create the plot you suggested (new Figure 12). In this plot the maximum and minimum temperature of the year corresponds to light and dark bands and the periodical oscillations in Mg, Li and Sr/Ca reveal their reliability as temperature proxies. Nevertheless, as strongly recommended by all the Referees, we decided to change Figure 9 in the revised text (new Figure 11), in order to show more clearly the relationship between B/Ca and seawater temperatures. We therefore plotted also seawater maximum and minimum temperature values with B/Ca mean values in long and short cells, respectively. As you can see in the resulting new Figure 11, temperature shows a very poor influence on B data.

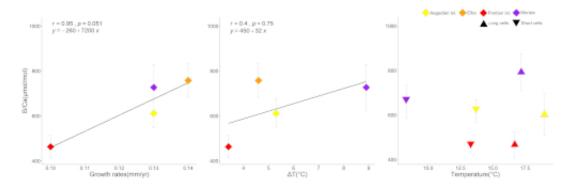


Figure 11: Correlation plots of growth rates and seawater temperature with B/Ca in *L. corallioides* samples analysed in this study. Spearman's coefficient r, the p-value and the line equation are given. Temperature variations (Δ T) correspond to the differences between the maximum and minimum temperature registered over 11 years of monthly reanalysis (ORAS5). The B/Ca means measured in long and short cells correspond respectively to the maximum and minimum temperature.

- **Comment:** Line 350: here a "poor correlation with temperature" is mention but it is a poor correlation with the magnitude of the seasonal temperature change that is plotted in fig9. Please change wording here.

Response: The Figure 9 (new Figure 11) has been changed as mentioned in the previous response.

- **Comment:** Line 354: This ending to the discussion unfairly represents the empirical calibration work that has gone into calibrating the δ^{11} B proxy in CA (e.g. Anagnostou et al., 2019). The controls on B/Ca in corals and foraminifera are also less well known compared to the strong seawater pH control on the δ^{11} B of these carbonates and therefore the use of δ^{11} B should not be discouraged for reconstructing past seawater pH

Response: Our comment was referring more specifically to the B/Ca; the original text was ambiguous, and it was therefore reworded.

- **Comment:** Line 363: There is no mention of Mg/Ca relationship in with deltaT in the main text, so it is strange to see this in the conclusions. It is again unclear why correlation to seasonal temperature range is important particularly when average Mg/Ca appears to correlate poorly with average temperature at these sites.

Response: The text has been modified for clarity. There is indeed no reference to a correlation in the main text, but rather a discussion about the fact that Mg/Ca means in the different sites mirror the amplitude of seawater temperature variations during the algal growth. The explanation of the importance of ΔT is in the comment above.

References

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