

Reply to Referee#3

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-AC3>, 2021

Dear Referee #3,

Thank you for your review. Our revised paper has been implemented following your suggestions and raw data have been included. Thank you also for your comments on the value of our results.

Hereafter I will reply to each of your comments.

- **Comment:** *Environmental data. The environmental data the authors use for the collected samples does not correspond with the date of collection, many of the samples have been compared with averages of temperature and pH for years after their collection dates:*

Aegadian Island – Collected 1993, temperature 1986 to 2017, pH 1993 to 2018, DIC 2019-2020

Elba Island – Collected 1990, temperature 1980 -1991, pH 1993 to 2018, DIC 2019-2020

Morlaix – Collected 1991, temperature 1980-1992, pH 1993 to 2018, DIC 2019-2020

Pontian Island – Collected 2017, temperature 1986-2017, pH 1993 to 2018, DIC 2019-2020

I understand the pH and DIC data are sparse, but there doesn't seem to have been any attempt to try and 'translate' these values to the collection time, or discuss the implications (besides lines 150-152).

Response: Temperature data have been extracted by 11 years of monthly reanalysis for each sampling site in the revised paper (the time interval considered for Aegadian Isl. has been corrected). The selected time interval (11 years) approximately covered the period of algal growth crossed by the laser transects and was the same for each site, for comparability. This choice has been better explained in the revised text (Section 2.3). As you pointed, carbonate system data are sparse instead, because of their reduced availability. The selected time interval of extraction (2019-2020) was the only period available for each sampling sites. We used two different biogeochemical models to extract pH and DIC data from the sampling sites, as described in the Section 2.3. pH data have been derived by CMEMS global biogeochemical hindcast. DIC data, which largely dictate the pH, have been extracted from CMEMS biogeochemical forecasts in the Mediterranean Basin and in the Atlantic Ocean. Even using different models, both extractions showed the same variations among sites, with the lowest values of both pH and DIC in Morlaix, and higher values in the Mediterranean sites (Aegadian Isl., Pontian Isl. and Elba). This suggests that the extracted data are not model's artefacts and allowed us to characterize the sampling sites. In conclusion, carbon data were used to characterize the different sites, allowing us to discriminate between the influence of carbon system parameters and

other factors on B/Ca. Temperature data, instead, allowed us to reconstruct the timeline of algal growth. The text has been implemented in the revised version.

- **Comment:** 'Raw' data. Like the other reviewers I miss the data of the individual samples and therefore it is difficult to make a fair assessment of many of the points raised by the authors. I don't know how many samples were actually analysed by the authors, is it one per site, or multiple. Likewise, how many measurements per sample. I would like to see the different elemental ratios per sample per site plotted, highlighting the measurements that represent the long or short cells. I would also plot the growth rate for each band (so that would be equal to length) as a shaded grey line behind.

Response: Raw data have been submitted to the PANGAEA repository. Moreover, a new table (Table 2) has been created to summarize the results of all element analyses in every sample. The analyses have been performed on one algal branch per site, as more clearly specified in the Materials and Methods section of the revised text. The laser transects consisted in 21-49 spots, which will be shown in the new supplement figure (Figure S1), if the Editor agrees. The new Figure 12 in the revised paper shows the elemental ratio variations across the algal thallus of the Morlaix sample, as well as temperature changes through warm and cold seasons. Light and dark bands have also been evidenced.

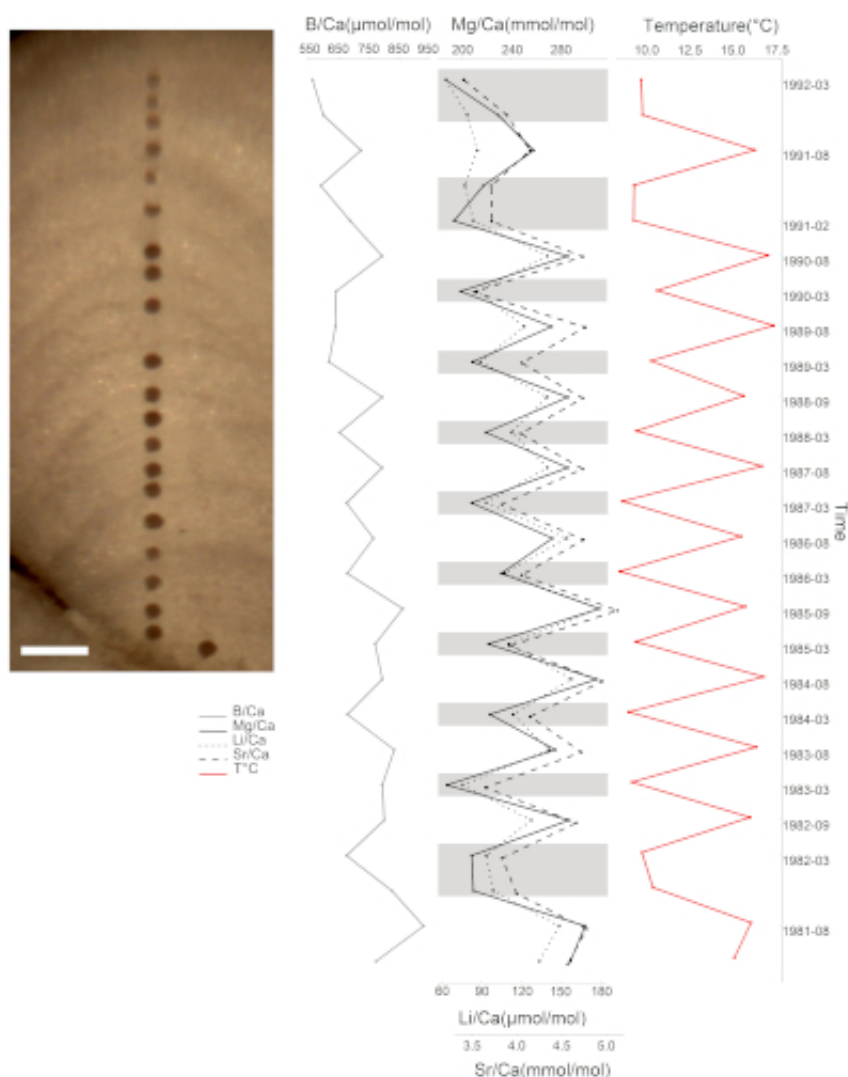


Figure 12 (new): Elements ratio of *L. corallioides* collected in Morlaix Bay (scale bar = 200 µm). Mg, Li and Sr/Ca show cyclic variations the same as the local seawater temperature. In the timeline, the coldest and the warmest months

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:** Growth rate vs DeltaT: Figure 9 seems to be the justification for the much of the title and abstract. I have no problem with four datapoints, if those data points incorporated error bars the showed the full range of the B/Ca. I do however wonder what DeltaT is supposed to represent as a variable, are the authors implying that seasonality controls the absolute value of the B/Ca? That is not testing temperature as the title suggests but the seasonal range in temperature (the datapoint at DeltaT = 9 would be the lowest average temperature site). I note that range of the B/Ca (from the boxplots in figure 8) also eclipses the y-axis of figure 9, the intra sample/site ranges from ~300 to ~950 B/Ca.

DeltaT. The authors use the range of temperature throughout and I am at a loss as to understand why you would compare a variable that is supposed to be a temperature proxy with the seasonality of the site. I could understand if the authors took the difference between max and min of the Element/Ca and compared it against DeltaT, but essentially the authors are comparing an absolute temperature with its supposed range.

Response: Error bars have been added to Figure 9 (new Figure 11). As explained also in the comments to the other reviewers, we believe that the seasonal temperature change (ΔT), which was calculated as the difference between the maximum and minimum seawater temperature registered during the algal growth, better characterized the sampling sites compared to absolute temperature, given their differences in depth and geographical regions. As an example, Elba and Pontian Isl. have temperature means of 15°C. Nevertheless, in Pontian Isl. (66 m depth), the temperature keeps more constant throughout the period ($\Delta T = 3^\circ\text{C}$), while in Elba there is a higher amplitude of temperature variations ($\Delta T = 5^\circ\text{C}$). The effects of these fluctuations around the optimum of algal growth would not have been highlighted by the mean temperature value. Also, the laser ablation resolution 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 to the cold and warm season, as done for the creation of the new Figure 12, as suggested. For this reason, as strongly recommended by all the Referees, we decided to change Figure 9 (new Figure 11), to show more clearly the relationship between B/Ca and seawater temperature. We therefore plotted also the maximum and minimum temperature values per site with B/Ca mean values in long and short cells, respectively. As you can see in the resulting new Figure 11, the poor relationship between temperature and B/Ca data is even clearer.

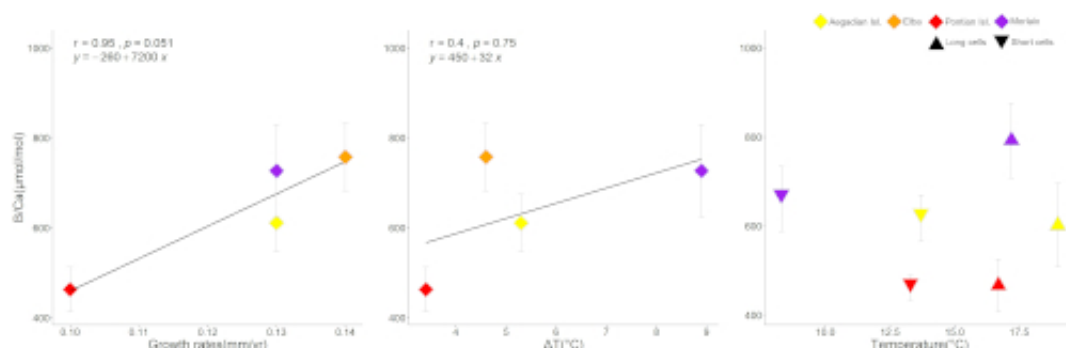


Figure 11: Correlation plots of growth rates and seawater temperature with B/Ca in *L. corallioides* samples analysed in this study. Spearman's coefficient r , the p -value and the line equation are given. Temperature variations (ΔT) correspond to the differences between the maximum and minimum temperature 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:** *Long and short cells. The authors use the Mg/Ca to check if the data is from long and short cells, does this not make Figure 5 completely irrelevant, i.e., the difference between the long and short is because they were split by Mg/Ca ('Long cells were identified as spots with high Mg/Ca and positioning on light growth bands. Conversely, short cells were located in dark growth bands and corresponded to low Mg/Ca ratio.'). The all Mg/Ca (i.e., long+short) vs sites shows (Figure 4) no difference, therefore the only rationale to show figure 5 would be to inform the reader what cut off values you used to define short and long cells.*

Response: The description of the method we used to distinguish between dark and light bands has been implemented for clarity. To make a reasonable distinction between long and short cells, we did not consider the data from the laser spots that had a doubtful interpretation. We thus discarded the data derived from spots positioned on faint bands and those referring to middle seasons, which have intermediate Mg/Ca values. By doing so, we preserved only the unequivocal information without any data manipulation.

- **Comment:** *Sampling at growth bands. Line's 165 and 135 'each growth band change which marked the transition between the cells usually produced in the warm season and those usually produced in the cold season,' you are targeting transitions between Cold and Warm Seasons. So does the growth band really represent the cold season or warm season in its entirety? If you sample the transition between cold and warm by definition you don't sample the extremes (which DeltaT would represent) but do you fully sample the temperature? If a growth band represents a season, comparing the average of X number of growth bands is not entirely comparable with the mean temperature of the site (you could sample 3 cold and 4 warm growth bands, i.e., W-C-W-C-W-C-W, and therefore your average element/ca signal would be biased).*

Response: Referring to your first observation, the longitudinal sections were performed along the maximum branch thickness. As you can see in Figure 2, we targeted the centre of each bands which ideally correspond to the peak of temperature registered in the warm/cold period. We thus have tried to avoid sampling the transition between adjacent bands, as far as possible by the method. As explained in the previous response, when comparing short and long cells the information coming from doubtful points of analyses have been discarded.

Concerning your second observation, we performed the statistical analyses on both the original dataset and a modified dataset, which included the results from the same number of light and dark bands. This corrected dataset had the same results in the statistical tests and did not highlight differences or other additional information.

- **Comment:** *Probably avoid abbreviating coralline algae as CA if you're talking about [Element]/Ca (e.g., line 13 'Mg, Sr, Li and B in the CA')*

Response: The text has been changed.

- **Comment:** *Line 21: "These pieces of evidence suggest that growth rates, triggered by the different ΔT and light availability across depth, affect the B incorporation in *L. corallioides*." – does this not contradict your title?*

Response: Multiple factors act together in influencing the element incorporation of the algae during their growth. Nevertheless, according to our interpretation there is a major control of growth rates, rather than temperature, on B/Ca. For example, in Morlaix, at very shallow depth (12 m), the apparent influence of temperature on B/Ca is more likely linked to growth rate changes due to the high seasonality of the site (expressed by the

high ΔT value). The text has been revised for clarity.

- **Comment:** Line 42: define acronym - MAS NMR

Response: The text has been changed.

- **Comment:** Line 83: "in calcifying species, including CA (Barker et al., 2005;" – perhaps split the references between those that did and did not use coralline algae

Response: The text has been changed.

- **Comment:** Line 92 and Line 100: A bit repetitive use of 'in this paper', perhaps at line 100 change to 'With our new measurements we aim to test whether...' to clarify objectives

Response: The text has been changed.

- **Comment:** Line 105: If grab samples were used, how sure are you they were collected living specimens. Also in general, is collection date supposed to be read as collected living at this date

Response: Living and non-living samples were discriminated directly on board after grabbing, by some of the authors (DB and VB) using visual analysis. The red pigments of the algae rapidly degrade in seawater after death, facilitating the distinction. The specimens used for this work were all alive at the time of collection.

- **Comment:** Line 120: Missing word - 'Hence once excluded [specimens?] belonging to the genus...'

Response: The text has been changed.

- **Comment:** Line 114- 122: Clarify and expand 'how' (i.e., traits and characteristics used) and 'why' identification was needed, in the 'why' justify species selection.

Response: Species identification was achieved by using classical morphometrical descriptors based on epithallial and perithallial cells observations on SEM images. Macromorphology, indeed, is not sufficient to discriminate between *P. calcareum* and *L. corallioides*. *L. corallioides* is a suitable species for the wide geographic scope of this work due to its presence in both Mediterranean and Atlantic waters. The text has been implemented in the revised paper.

- **Comment:** Line 124: replace included with embedded/fixed/placed

Response: The text has been changed.

- **Comment:** Line 125: replace 'kept drying' with 'left to dry'

Response: The text has been changed.

- **Comment:** Line 125: 'included branches' – replace with 'treated branches'

Response: The text has been changed.

- **Comment:** Line 144 – 149: why 11 years?

Response: The selection of this time interval has been explained in the main response above and has been implemented in the revised text.

- **Comment:** Line 160: "The obtained values, expressed in linear extension over year (mm/yr), were crossreferenced with Mg/Ca results in order to check for the correspondence of Mg peaks with growth band helpful in highlighting faint bands and to achieve a more reliable estimate of the algal growth" – is this not circular? I note this is further expanded at lines 216-217 to 'Long cells were identified as spots with high Mg/Ca and positioning on light growth bands. Conversely, short cells were located in dark growth bands and corresponded to low Mg/Ca ratio'

Response: The method used to discriminate between long and short cells has been implemented in the revised text. A detailed explanation is in the response to your main point above.

- **Comment:** Line 163-165: what is the difference between the growth rates of the light and dark/long and short cells?

Response: It was not possible to infer the growth rates of single bands with an acceptable error. We measured algal growth rates by dividing the laser transect to the number of years of growth. This method implies a non-negligible error margin and increasing in resolution as you suggest would increase the error as well and the data would lose reliability.

- **Comment:** Line 200: "Both Li/Ca and Sr/Ca records had positive correlations with Mg/Ca in our samples" is this not because it's a closed sum? With an elemental ratio there is only so much substitution available in the calcite lattice, so what do these correlations show?

Response: The correlations show a covariance between Li and Sr/Ca and Mg/Ca, which is mainly controlled by temperature. This suggest that also Li and Sr/Ca are temperature dependent. This is supported by the new Figure 12 in the revised text which shows the seasonal variations in these element ratios, related to the seasonal temperature changes. The same trend is not observed for B/Ca, in fact, the correlation between B/Ca and the temperature proxies is not supported.

- **Comment:** Line 277: 'In order to provide high-resolution geochemical data on long and short cells separately, we considered only the results from the spots where the positive/negative Mg/Ca peaks correspond respectively to the light/dark bands resulting from the image analyses.' + Line 281-282: 'In the sample from Elba the growth bands were not clearly visible, preventing the analyses of trace elements on long and short cells separately.' These should occur earlier at sections 2.4 growth and 2.5 data.

Response: The text has been changed.

- **Comment:** Line 299: Is 60m deepwater?

Response: The term "deep" has been corrected throughout the revised text.

- **Comment:** Line 300: 'The results of the statistical analyses on Mg/Ca evidenced a strong relationship with the seawater temperatures extracted from ORAS5 (Table 2), as expected' – Table 2 is only the temperature so we as readers don't know if it is a strong relationship plus the extracted environmental variables in places do not correspond with the sampling dates. Plot the data not as a box plot (fig 4) but as a scatter plot with the full range of values as error bars.

Response: Concerning your first observation, the revised text has been implemented for clarity. We created the scatter plot you mentioned; however, we believe that plotting the

data as a scatter plot would confound the reader and the data shown in Figure 4 are clearer as a box plot. Moreover, we performed a non-parametric test to evaluate the differences in Mg/Ca among sites, and the box plot showing the median values is more appropriate for these kind of statistics.

- **Comment:** Line 307: 'Temperature covaries with irradiance and both correlate to seasons, which influence primary production, respiration and calcification in *L. corallioides*' - Do you (a) think that there is much irradiance change between 37 and 48N? There is roughly an 2 hr difference in length of day between 30 and 50 N. And (b) temperature lags irradiance, its why September is still relatively warm (as it takes time for the sea to 'warm up' due to seawaters heat capacity) along Europe's coasts.

Response: This sentence was cited from Martin et al. (2006). We understood your doubts about irradiance and the text has been changed for clarity. Indeed, the difference between Morlaix and the Mediterranean sites relies mainly on seasonal fluctuations in temperature (as highlighted by ΔT variations), and our discussion was not referring to irradiance.

- **Comment:** Line 338: You can calculate the potential difference in light between 40 and 60 m to actually determine if irradiance matters, like so: The light at depth Z is equal to the light at surface multiplied by e to the power $-kz$, where z is the depth and k is an extinction coefficient which varies from basin to basin.

Response: This would be very interesting, and we thank you for your suggestion. Nevertheless, it would be beyond the aim of our present work.

- **Comment:** Line 399: Data availability. Should be deposited in a repository rather than available on request (https://www.biogeosciences.net/policies/data_policy.html).

Response: Data have been submitted to the PANGAEA repository.

- **Comment:** Line 414: Move 'Environmental data were provided by E.U. Copernicus Marine Service Information' to Data availability, include the url as well as the url of ORA S5.

Response: The change has been made.

- **Comment:** Figure 2B: The coralline algae is branching so I wonder how do you determine which is the oldest/youngest and therefore corresponds to your environmental data. For example, if you go up from between the 9th and 10th spots from the left, two branching growth lines meet, one sits slightly above the other (so that branch formed after). And because its branching does that not impact growth rate? I.e., what if the algae invested more time in branching than lengthening a branch?

Response: Your observation is correct. The algae may grow in different directions and thus their growth patterns are often complicated. In this work, we followed one single growth direction per transect. The discontinuity you highlighted in Figure 2 marks a change in direction and of course the alga could have invested time in branching as you suggested. Our measurements of the growth rates, as responded above, have a certain margin of error which is also influenced by the complexity of the algal growth patterns. This is also the reason why we cannot reasonably measure the growth rates of single bands as mentioned above.

- **Comment:** Figure 2B: Because the growth lines are convex how is growth measured, the longest point between two growth lines or fixed? Or more pertinently if the laser ablation track is used to define the size, how were the laser ablation sites positioned?

Response: Longitudinal sections were cut across the maximum thickness of the branch. The laser transects crossed the growth bands by the mid length, as you can see in Figure 2.

- **Comment:** *Figure 5: If Mg/Ca is used to define the long and short cells this is not an independent test.*

Response: The method used to distinguish long cells from short cells has been implemented in the revised text, as explained in the major points above. Long and short cells are not dependent on Mg/Ca by definition, but they resulted to be so (Figure 5).

- **Comment:** *Figure 9: $p = 0.051$, technically that is above $p < 0.05$.*

Response: Correct. We did not mention a statistically significant correlation given the borderline value, but the positive relationship shown in Figure 9 (new Figure 11) is noteworthy.

- **Comment:** *Figure 9. The two lowest datapoints with B/Ca values of 460 and 610 look like they are subtly offset between the left and right panels*

Response: Figure 9 (new Figure 11) has been changed.

References

Martin, S., Castets, M.-D. and Clavier, J.: Primary production, respiration and calcification of the temperate free-living coralline alga *Lithothamnion corallioides*, Aquat. Bot., 85, 121–128, doi:10.1016/j.aquabot.2006.02.005, 2006.

Please also note the supplement to this comment:

<https://bg.copernicus.org/preprints/bg-2021-21/bg-2021-21-AC3-supplement.pdf>