Biogeosciences Discussions

Biogeosciences Discuss., author comment AC2
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Reply to Referee#2

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-AC2, 2021

Dear Referee #2,
Thank you for your comments. Our paper has been implemented and improved following your suggestions. English has also been checked throughout the text. We also appreciate that you have recognized the importance and novelty of our results.
Hereafter I will reply to each of your comments.

- ***Comment:*** *The authors use samples collected from different time intervals (1990–2017), but does not discuss how global warming changed water temperature and how much this consideration influence their discussion. Similarly, they discuss relationship between B/Ca and pH/DIC, but correction due to anthropogenic CO_2 invasion (ocean acidification) is not considered.*

Response: The point that you mentioned is certainly interesting but goes far beyond the objectives of this work and its methodological ability. The error attributable to the offsets between the proxy and the actual temperature/pH would probably be higher than the magnitude of variations occurred in that period because of climate change.

- ***Comment:*** *There is no explanation about analytical precision of LA-ICPMS used in this study. I wonder how reliable and reproducible their analysis is?*

Response: More details regarding the analytical precision of the method have been added in the Section Materials and Methods. Accuracy and precision were higher than 4% and 8% for NIST 612 and Ca standards respectively.

- ***Comment:*** *There is no figure on Mg/Ca variation and its comparison to dark/light growth bands for each sample, which likely aids readers to understand the discussion. For example, the authors argue that the Aegadian sample records 10 years of growth, but the reader cannot see this.*

Response: A figure showing Mg and other elements oscillations across the algal thallus of the sample collected in Morlaix has been added (new Figure 12), evidencing light and dark growth bands variations. A new figure showing the laser transects crossing in longitudinal section the samples collected in the Mediterranean sites will be added as .pdf Supplement (new Figure S1), if the editor agrees.

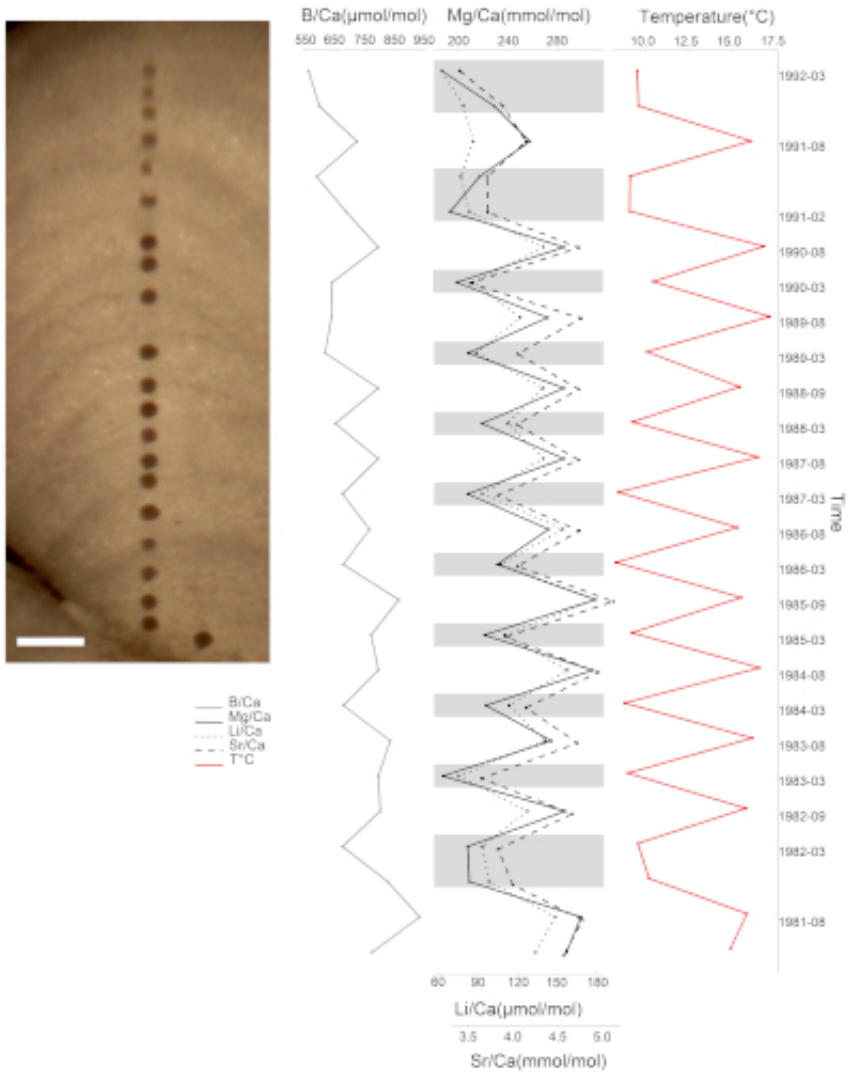

Figure 12 (new): Elements ratio of *L. corallioides* collected in Morlaix Bay (scale bar = 200 µm). Mg, Li and Sr/Ca show cyclic variations the same as the local seawater temperature. In the timeline, the coldest and the warmest months have been reported, which correspond to dark and light bands of growth. 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:*** *The authors argue that Li/Ca and Sr/Ca of CA are temperature proxy, because there is linear relationships between Li/Ca and Mg/Ca as well as between Sr/Ca and Mg/Ca, but I don't agree this. There is no figure supporting a significant correlation between Mg/Ca and temperature. The relationship between Mg/Ca and temperature is a key of the discussions of this manuscript. A box plot in Fig. 4 shows no significant differences among sites (N = 4). Also, there could be a pseudo-correlation between Li/Ca and Sr/Ca with temperature, because many environmental factors vary in the same phasing (e.g., irradiance, temperature, growth rate).*

Response: The reliability of Mg/Ca as a temperature proxy in calcareous red algae has been recognized since the mid-1990s (Chave and Wheeler, 1965; Moberly, 1968; Henrich et al., 1996; Halfar et al., 2000; Kamenos et al., 2008). We do not have evidence to cast doubts on this hypothesis. On the contrary, the plot we added in the revised version (new Figure 12), as you and the other reviewers suggested, clearly shows the relationship

between the seasonal oscillations in Mg, Li and Sr/Ca ratios and the seawater temperature, across light and dark bands. Moreover, Mg/Ca mean values across sites, although they are not statistically significant, decrease as the magnitude of temperature fluctuations during the algal growth decreases. What you were referring to as a "pseudo-correlation" could be the case of any element incorporated by the alga, it is true. The limit of performing geochemical analyses on wild-grown algae is the impossibility to control all the environmental factors during algal growth, contrary to what is done during culture experiments. On the other hand, recognizing patterns in wild-grown specimens is only possible when, despite variability, an overarching control can be observed, which is the strength and the novelty of our observations.

- ***Comment:*** *"p" of pKb and pCO2 should be italic.*

Response: The text has been corrected as you suggested.

- ***Comment:*** *Line 32: What is "normal pH conditions"?*

Response: The text has been changed to "typical surface seawater conditions".

- ***Comment:*** *Line 35–36: The notion that "Seawater isotopic composition $\delta^{11}Bsw$ is 39.61‰ (Foster et al., 2010) and varies with the isotopic composition of $B(OH)_3$ and $B(OH)_4^-$" depend on timescales concerned.*

Response: In the text it has been specified that we were referring to current seawater isotopic composition (Foster et al., 2010).

- ***Comment:*** *Line 41: The expression that TA and DIC "are closely related to the $\delta^{11}B$ of the borate" is wired. Yes, DIC and TA is related to pH, and pH can be indirectly estimated from $d^{11}B$ of some kind of calcium carbonate.*

Response: Line 40: "The pCO_2, the seawater $[CO_3^{2-}]$, pH and $[B(OH)_4^-]$ are mainly controlled by the balance between the total alkalinity (TA) and the dissolved inorganic carbon (DIC) and are closely related to the $\delta^{11}B$ of the borate". The subject of this sentence was "pCO_2, the seawater $[CO_3^{2-}]$, pH and $[B(OH)_4^-]$", not TA and DIC. However, the text has been modified in the revised version for clarity.

- ***Comment:*** *The notion that "the boron-to-calcium ratio (B/Ca) is informative about past seawater CO_3^{2-} concentrations (Yu and Elderfield, 2007; Yu et al., 2007; Rae et al., 2011)" is only true to foraminifera. As for reef building corals, B/Ca is related to other carbonate parameters (see Holcomb et al., 2016).*
Holcomb, M., DeCarlo, T. M., Gaetani, G. A., & McCulloch, M. (2016). Factors affecting B/Ca ratios in synthetic aragonite. Chemical Geology, 437, 67–76. https://doi.org/10.1016/j.chemgeo.2016.05.007

Response: Thank you for the reference. The relationship between B/Ca and CO_3^{2-} had been tested empirically in foraminifera, as you pointed. Holcomb et al. (2016) performed synthetic aragonite precipitation experiments, also confirming the suitability of B/Ca as a proxy of $[CO_3^{2-}]$. Scarce data exist on coralline algae, which are composed by high-Mg calcite, and there is no specific mention about B/Ca coupled with CO_3^{2-} data. This has been specified in the text. In culture experiments of the coralline alga *Neogoniolithon* sp., Donald et al. (2017) found a negative effect of DIC on B/Ca.

- ***Comment:*** *Line 58: What is "indeterminate growth". Please be more quantitative. Also, "no ontogenetic trend" of what?*

Response: The concept has been clarified in the text. The term "indeterminate growth" is

commonly used when referring to the longevity of coralline algae (Adey, 1965; Frantz et al., 2005; Halfar et al., 2008). Their growth trend does not slow down asymptotically with age, following the so-called "ontogenetic trend" as bivalves do. Coralline algae thus preserve the resolution of the geochemical signals even in the later stages of growth.

- ***Comment:*** *Lines 70–73: I could not understand this line "Achieving the best reliability of geochemical proxies for climate reconstructions is indeed crucial, which drives a growing interest on multiple approaches, by considering multi-proxies for a single environmental factor (D'Olivo et al., 2018; Zinke et al., 2019; Cuny- Guirrec et al., 2019), as well as the influence of multi-factors on a single proxy (Kaczmarek et al., 2016; Donald et al., 2017)."*

Response: The text has been rephrased for clarity, as following: "To achieve the best reliability of geochemical proxies for climate reconstructions, a growing interest has arisen on multiple approaches, by considering multi-proxies for a single environmental factor (D'Olivo et al., 2018; Zinke et al., 2019; Cuny-Guirrec et al., 2019). Moreover, it is important to recognize the influence of multiple factors on a single proxy (Kaczmarek et al., 2016; Donald et al., 2017)."

- ***Comment:*** *Line 76: "positive correlations" between what?*

Response: "positive correlations" has been deleted. Indeed, the correlations we were referring to are specified in the next sentence.

- ***Comment:*** *Line 77 "B" should be "B/Ca" or "[B]"?*

Response: B/Ca, it has been changed in the text.

- ***Comment:*** *Line 79 "Foslie 1898" should be (Foslie)?*

Response: *Clathromorphum compactum* (Kjellman) Foslie 1898 is the correct nomenclature. Please check Guiry & Guiry AlgaeBase (https://www.algaebase.org/). The use of parentheses has specific meaning in nomenclature.

- ***Comment:*** *Line 78: Is "sea surface temperature (SST)" relevant to this study? Temperature is better here.*

Response: The text has been modified.

- ***Comment:*** *Line 83 About "calcifying species". Mg/Ca is a paleo-thermometer with regard to calcifying organisms that have calcite crystal form such as foraminifera and CA. Sr/Ca, not Mg/Ca, is paleo-thermometer in coral skeleton.*

Response: We agree with your comment. The papers cited in the sentence refer to coralline algae and we have written explicitly "calcite lattice".

- ***Comment:*** *Line 82–87: I wonder why the authors don't mention Li/Mg temperature proxy (especially for coral) here, because which is commonly used.*
K. Cuny-Guirriec, E. Douville, S. Reynaud, et al., Coral Li/ Mg thermometry: Caveats and constraints, Chemical Geology, Volume 523, 30 September 2019, Pages 162-178

Response: Mg/Li calibration did not improve the Mg/Ca or Li/Ca temperature relationship in previous empirical studies on coralline algae (Caragnano et al., 2014; 2017), as for corals instead. To test this proxy, we added Mg/Li results in the revised text. We plotted Mg/Li data with the results from Anagnostou et al. (2019) on cultured coralline algae *Clathromorphum compactum* (new Figure 6). Moreover, we also plotted Mg/Li oscillations

through time, which showed a poor relationship with seawater temperature (new Figure 7), contrary to the other temperature proxies (new Figure 12).

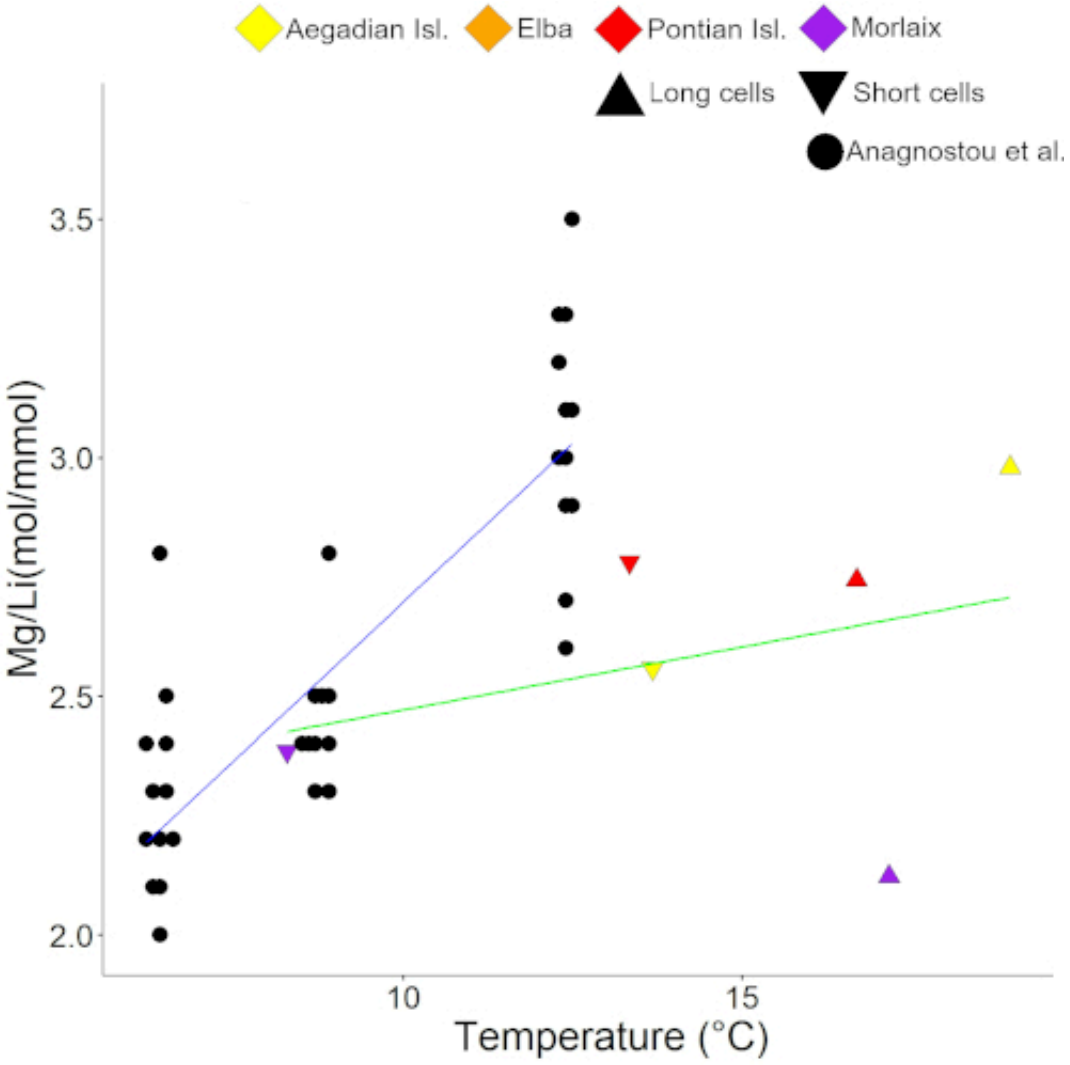

Figure 6 (new): Correlation plot between Mg/Li and seawater temperature. Data are shown for cultured *C. compactum* (Anagnostou et al., 2019) and *L. corallioides* (this paper). *L. corallioides* results are shown separately in long and short cells, per sampling site.

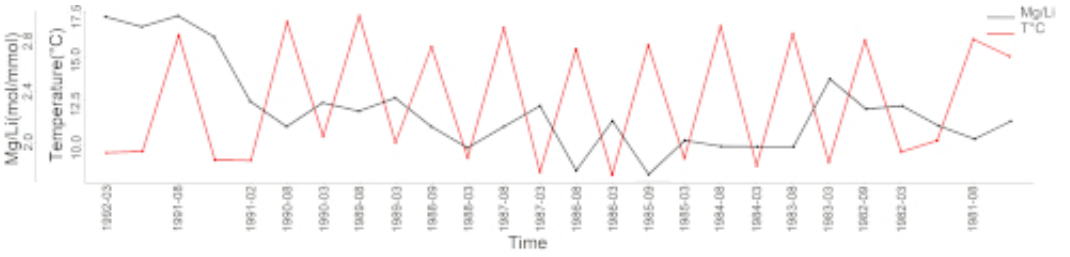

Figure 7 (new): Mg/Li ratio of *L. corallioides* collected in Morlaix Bay. Note the lack of cyclic variations in Mg/Li results. In the timeline, the coldest and the warmest months have been reported. Mg/Li in the missing bands have been calculated as the means of the values measured in warm and cold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

Monthly means of seawater temperature have been extracted by ORAS5 reanalysis.

- ***Comment:*** *Lines 92–95: Please revise these sentences: "In this paper, we measure by LA-ICP-MS for the first time the temperature proxies (Mg/Ca, Sr/Ca, Li/Ca) and B/Ca in the nongeniculated CA* Lithothamnion corallioides *(P. Crouan & H. Crouan) P. Crouan & H. Crouan 1867 collected from different geographic settings and depths across the Mediterranean Sea and in the Atlantic Ocean."*

Response: We have revised the sentence.

- ***Comment:*** *Line 97: Remove "Here, "*

Response: The text has been modified.

- ***Comment:*** *Line 100: Please revise these sentences: "In this paper, we test the B/Ca ratio versus the temperature proxies and the growth rates in order to evaluate their effects on B incorporation, which, indeed, could distort the B signal used for paleoclimate reconstructions."*

Response: The sentences have been revised.

- ***Comment:*** *Line 116–122: Please revise these sentences: "Morphological identification was based on Adey & McKibbin (1970), Irvine & Chamberlain (1994), and other information about maerl species distribution provided by Carro et al. (2014) and Melbourne et al. (2017). The*
samples selection started from a much wider collection than the one eventually selected for the chemical analyses. Particularly, the Atlantic sample (Morlaix) was used as voucher specimen for the subsequent identification of the Mediterranean samples,
since Phymatolithon *spp. and* L. corallioides *are the major components in the Atlantic maerl (Hall-Spencer et al. 2010; Carro et al., 2014). Hence, once excluded the belonging to the genus* Phymatolithon*, the Morlaix sample identified as* L. corallioides *was used as a reference for the most reliable identification of the other Mediterranean samples."*

Response: The sentences have been revised.

- ***Comment:*** *Line 124: How large "algal branches" were? I guess less than 5 mm from Fig. 2.*

Response: Correct, the algal branches were to the order of 5 mm as shown in Figure 2.

- ***Comment:*** *Line 125 What is "treated samples"? Does this mean that the samples were maintained for 24h in a resin under the vacuum (drying)?*

Response: Correct, the treated samples are the included algal branches. The text has been modified for more clarity.

- ***Comment:*** *Line 132: Should be "according to", instead of "in agreement with"?*

Response: The text has been changed.

- ***Comment:*** *Line 130 (Section 2.3) I wonder the authors did pre-ablation of CA sample surface? The usage of distilled water instead of MQ water is enough to remove surface contamination? Especially, boron is easily contaminated from the environment.*

Response: We did not perform pre-ablation. However, during processing the signal was

excluded in the first part, as potentially affected by contamination. This has been specified in the Materials and Methods section.

- ***Comment:*** *Lines 144–149: Please revise these sentences: "In the absence of in-situ environmental data, the seawater temperature data have been extracted by at least 11 years of monthly reanalysis spanning 1980-2017 from ORAS5 (Ocean ReAnalysis System 5), at 0.25-degree*
horizontal resolution (Zuo et al., 2019). The nearest sea point of the three-dimensional numerical grid was considered for each sample location. Details of the time interval considered for each sampling site are shown in Table 2. Minimum, maximum and mean values, as reported in Table 2, refer to the temperature at sampling depth and have been measured on the entire time interval for which the data have been extracted"

Response: The sentences have been revised.

- ***Comment:*** *Lines 150–158: Please revise these sentences: Carbon data in each sampling site have also been extracted. They were not available in the same time interval of temperature data. Nevertheless, the seasonal variations occurring in the extracted period have allowed the characterization of the sampling sites. Monthly mean seawater pH has been derived by the CMEMS (E.U. Copernicus Marine Service Information) global biogeochemical hindcast spanning 1993-2018, at 0.25-degree horizontal resolution. Monthly means of DIC in 2019 and 2020 have been extracted by CMEMS biogeochemical analysis and forecasts for the Mediterranean Sea, at 0.042-degree horizontal resolution (Salon et al., 2019; Bolzon et al., 2020). In the Atlantic site, monthly means of DIC were derived from CMEMS IBI biogeochemical forecasts, at 0.028-degree horizontal resolution covering the years 2019-2020. Minimum, maximum and mean values of DIC, as reported in Table 2, refer to sampling depth and have been measured on the entire time interval of extraction." Also, I have a great concern here. Surface seawater DIC is changing over time due to CO2 invasion from the atmosphere. Thus, the authors need to correct this influence when comparing pH and DIC among sampling sites (Lines 189–192).*

Response: The sentences have been revised. pH and DIC data refer to the sampling depth in each site (12, 40, 45 and 66 m depth), not to the surface. We do not believe that the influence of anthropogenic CO_2 could be relevant to our analyses, due to the resolution of the method as explained above for climate change issues.

- ***Comment:*** *Line 191 and Table 2: An more common unit of DIC is μmol/kg, not mol/m^3.*

Response: The unit has been converted, thank you for the suggestion.

- ***Comment:*** *Line 160–161: I wonder why they made estimation of linear growth rate in this m manner, because this can be done by using image software such as Image J using Fig. 2b. Is it due to the fact that CA has many "faint bands" and variation of Mg/Ca is more reliable to see summer/winter seasonality?*

Response: Correct, the estimation of growth rates under light microscope allowed us to distinguish seasonal bands with a higher degree of confidence, since these bands are not obvious in the images (Figure 2 and new Figure S1). Moreover, once confirmed the positive correlation between temperature and Mg/Ca variations, Mg/Ca peaks were used to double-check the correspondence of the years, improving reliability.

- ***Comment:*** *Line 179: Please explain what "ΔT" stands for in the main text, not in the Table caption.*

Response: The definition of ΔT has been added in the Materials and Methods (Section

2.3). Thank you for your comment.

- ***Comment:*** *Lines 181–189: Does the second decimal place in water temperature have any meaning?*

Response: The approximation has been changed to one decimal place.

- ***Comment:*** *Line 208 The word "widest oscillation" sounds wired. Please rephrase it.*

Response: The sentence has been rephrased.

- ***Comment:*** *Lines 216–217: It's a circular argument, because the authors use variation of Mg/Ca to distinguish light and dark bands.*

Response: The description of the method we used to distinguish between dark and light bands has been implemented, because the text was probably misleading. In order to make a reliable distinction between long and short cells, we discarded intermediate Mg/Ca values that probably refer to middle seasons, as well as data from spots positioned on faint bands.

- ***Comment:*** *Lines 233–234: Is there any statistics showing no correlation between B/Ca and temperature?*

Response: B/Ca in the Mediterranean samples had no significant correlations with temperature proxies Mg, Li and Sr/Ca, resulting in a Spearman's p value > 0.05, as cited in the text. This is also true for the correlation between B/Ca and seawater temperatures in the different sites. We changed 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 the maximum and minimum temperature values registered in the extracted time interval 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. Moreover, the new Figure 12 shows a poor relationship between B/Ca oscillations and seasonal temperature variations even in Morlaix, contrary to what observed for Mg, Li and Sr/Ca.

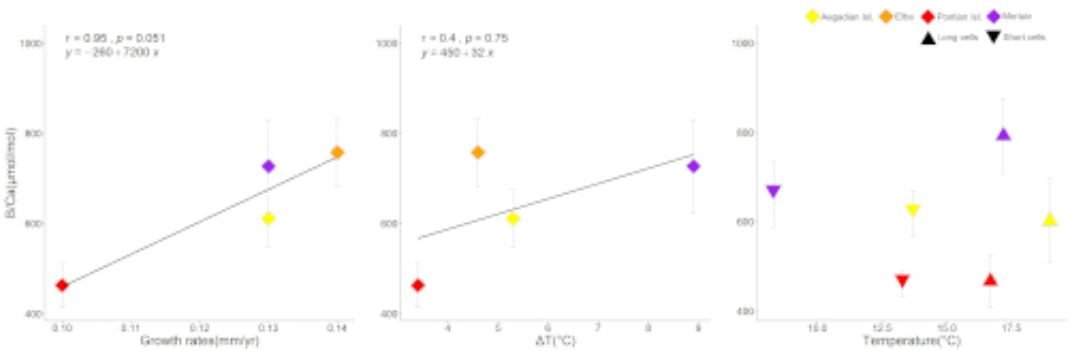

Figure 11: Correlation plots of growth rates and seawater temperature with B/Ca in *L. corallioides* samples analysed in this study. Spearman's coefficient r, the p-value and the line equation are given. Temperature variations (ΔT) correspond to the differences between the maximum and minimum temperature 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:*** *Line 271–272: Positively correlated, but insignificant, right? (Probably due*

to a small sample number: N = 4)

Response: Yes, the correlation is strongly positive with a "borderline" p value of 0.051. As you wrote, this could be related to the small sample number, but we believe it is noteworthy.

- ***Comment:*** *Lines 288–295: I could not understand this lines. How important the B/Ca range comparison between cultured and naturally obtained (wild) CAs is? There is no mention about correlation between B/Ca and pH/pCO_2/DIC, etc.*

Response: What we want to stress is precisely the paucity of data on B/Ca in coralline algae. As you pointed, the papers we cited to compare our data with the literature refer to cultured algae. Using LA-ICP-MS could probably have contributed to the wider range of B/Ca measured in *L. corallioides*, compared to the bulk analyses performed by Donald et al. (2017) and Anagnostou et al. (2019), as suggested by Referee#1 and specified in the text. However, there are no other data on wild-grown coralline algae collected across sites with different environmental settings, neither other geochemical analyses on *L. corallioides*. The correlation between B/Ca and carbonate system parameters is discussed in the following paragraphs (Line 317) and has been further implemented.

- ***Comment:*** *Line 297: What does "the preservation state of mineral structures" influence trace element/Ca ratio of CA? Does this mean that they can be altered in a live-caught specimen?*

Response: Early diagenesis during growth stages in rhodoliths could compromise the geochemical signals and therefore a prior evaluation of the conservation status of the alga should be important.

- ***Comment:*** *Lines 299–301: I'm not convinced by this argument, because there is no figure supporting a significant correlation between Mg/Ca and temperature. A box plot in Fig. 4 shows no significant differences among sites. Based on a correlation of Mg/Ca with temperature (not shown), the authors argue that "For the first time, we confirmed here the reliability of the temperature proxies Li/Ca and Sr/Ca on a deep-water Mediterranean CA"? I wonder how reliable these proxies as thermometer are. Similarly, there is no evidence that "The results of the statistical analyses on Mg/Ca evidenced a strong relationship with the seawater temperatures extracted from ORAS5 (Table 2), as expected."*

Response: Mg/Ca has successfully been applied as temperature proxy in coralline algae and we have no reason to doubt its reliability. The revised paper also includes a new plot showing the cyclic variations of Mg, Li and Sr/Ca through time which are evidently related to seasonal temperature variations (new Figure 12). Moreover, as responded in the comments above, even if the differences in Mg/Ca shown in Figure 4 are not statistically significant, Mg/Ca mean values decrease as the magnitude of temperature fluctuations during the algal growth decreases.

- ***Comment:*** *Lines 311–313: There could be a pseudo-correlation between Li/Ca and Sr/Ca with temperature, because many environmental factors vary in the same phasing (e.g., irradiance, temperature, growth rate). Classically, Mg/Ca of reef-building corals had been regarded as temperature proxy (now we know it is not). See, for example: Inoue M., Suzuki A., Nohara M., Hibino K. and Kawahata H. (2007) Empirical assessment of coral Sr/Ca and Mg/Ca ratios as climate proxies using colonies grown at different temperatures. Geoph. Res. Lett. 34, L1261. https://doi.org/10.1029/ 2007GL029628. B/Ca is also well co-related with Mg/Ca (Fig. 6), but not regarded as temperature proxy, according to the authors.*

Response: As we already discussed in the major point above, we cannot exclude the influence of multiple factors on elements incorporation, which is likely indeed. Our samples belong to calcareous red algae (coralline algae) that have obvious major differences with the physiology and mineralogy of corals. They have been collected in nature and therefore we could not control all the environmental factors involved during algal growth. Nevertheless, Li and Sr/Ca correlation with temperature is evidenced in the new plot showing seasonal oscillations across light and dark bands (new Figure 12). B/Ca results instead could not support its application as temperature proxy because its correlation with Mg/Ca is only significant in the sample from Morlaix, the shallowest site, highly influenced by seasonality (as shown by the highest temperature fluctuations). Also, in Morlaix B/Ca variations during the algal growth did not reflect the seasonal temperature changes (new Figure 12), contrarily to the proper temperature proxies.

- ***Comment:*** *Line 321: 8.32!?*

Response: The text has been corrected.

- ***Comment:*** *Line 326: pH and DIC "results"? The authors did not analyze seawater but just extracted data from the database.*

Response: The text has been changed referring specifically to extracted data.

- ***Comment:*** *Line 351: The title of this paper is based on the fact that there is "a closer relationship with growth rate rather than temperature" even though there is no statistical significance?*

Response: Figure 9 (new Figure 11) shows a noteworthy positive relationship between B/Ca and growth rates. We suggest that the different growth rates could have contributed to the B/Ca differences among sites that could not be justified by the differences in carbonate system parameters and are not related to temperature either.

- ***Comment:*** *Line 356: Does the authors would like to mention possibility of local modification of boron isotopic composition of seawater?*

Response: Foster et al. (2010) measured a mean seawater $\delta^{11}B$ of 39.61 ‰ from several ocean basins and found no significant or systematic variations of $\delta^{11}B$ with depth, salinity, or temperature, in agreement with its long residence time in seawater and despite the large range in salinity (32/38 psu) and temperature (-0.3/25.9 °C) considered. We therefore have no reason to believe in a modification of seawater boron isotopic composition among our sampling sites.

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