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Biogeosciences Discussions

Interactive comment on "Light and temperature effect on δ^{11}B and B/Ca ratios of the zooxanthellate coral *Acropora* sp.: results from culturing experiments" *by* D. Dissard et al.

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Herewith we submit the revised version of the manuscript entitled "Light and temperature effects on δ11B and B/Ca ratios of the zooxanthellate coral Acropora sp.: Results from culturing experiments" We appreciate the effort the reviewers put into our manuscript, which greatly benefitted from their comments. Each of their comments was addressed separately.

Answer to anonymous referee 1

Comment 1: Dissard et al. cultured Acropora sp. under 3 different temperatures and two different light conditions, to study the effects of temperature and light on the boron

isotope (d11B) and B/Ca proxies. They observe increasing d11B and B/Ca at higher temperatures, which they interpret as a temperature effect on both proxies, and observe relatively lower d11B and B/Ca at 400 μE compared to 200 μE. This data set is interesting but the data presentation and evaluation shows several shortcomings. First, in order to gauge the reproducibility of the geochemical analyses, it would need to be stated whether replicate analyses were splits of homogenized coral samples or true replicates of separate coral nubbins. Table 3 (now table 4 in the manuscript) provides average data of replicates and standard deviation of those averages. Those uncertainties are often much larger than the stated external uncertainty of d11B measurements (0.25‰ and given the low data density, they should be given as 2 sd, or better the actual data of individual replicates should be provided. Once that is done, I suspect it will become clear that the supposed temperature effect on d11B is not signiïňĄcant.

Answer: Reproducibility is based on true replicates of separate coral nubbins (2 replicates for each experimental condition, except for value 400, 28, where three replicates were considered). This is now clearly stated in the manuscript: "II.4. Geochemical measurements. For each experimental condition, elemental and isotopic measurements were performed on two replicates of separate coral nubbins incubated in the same culture conditions, except for condition 400, 28, where values presented are the average of three replicates." Because average data of "true" replicates are considered, standard deviation can happen to be larger than the analytical external uncertainty of 0.25 ‰ for d11B measurements (now SD presented in table 4 are combined SD of analytical uncertainties + difference between different replicates, for more details see answer to comment 4). In addition, as suggested by the reviewer, the actual data of individual replicates are now presented on Table 4. It should be noted here that values presented for each "true" replicates are an average of three measurements performed on the same solution (data not shown as analytical reproducibility should never be considered as replicates). The impact of light and temperature on Acropora sp. boron isotopic composition and boron concentrations remain unchanged.

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Comment 2: However, even if there was a true difference in δ11B between the different temperature treatments, the authors should consider the temperature-dependent changes in the pKB value, which decreases from 8.63 at 22 âÛęC to 8.56 at 28âÛęC. They reïňĆect on this brieïňĆy in Section 4.2.1 but only to generally compare the apparent pH offset between the site of calciïňĄcation and seawater-pH. Because that pH-offset is essentially constant between the different treatments (0.36, 0.40, 0.39 and 0.33, 0.36, 0.35 at 200 and 400 μE, respectively), those values seem incompatible with a signiïňĄcant temperature effect as suggested by the authors.

Answer: The reviewer is right when mentioning that it is important to consider the temperature-dependent changes in the pKB values as these value vary from 8.62, 8.58 to 8.55 at 22, 25 and 28ËŽC, respectively (salinity 38). In fact, these changes in pKB per temperature treatment were already taken into account when calculating the different pH-offsets. We now clearly stated in the manuscript section 4.2.1., that pKB values were corrected for the different temperature conditions: "The δ11B values measured in this study plot significantly above the curves and correspond to an increase in pH of the site of calcification of about 0.36, 0.40 and 0.39 pH units under LL, and 0.33, 0.36 and 0.35 pH units under HL, for 22, 25 and 28 ËŽC, respectively (δ11Bsw = 39.61‰ BT = 416 μM and pKB corrected for temperature and salinity using Dickson, 1990)." The values of the different pH-offset reported for different temperature conditions and calculated subtracting measured pH to δ11B derived pH of the site of calcification (0.04 and 0.03 between 22 and 25ËŽC, for LL and HL, respectively), are perfectly compatible and are in the same order of magnitude than the one calculated using the δ11B increases between the different temperature condition (increase of 0.03 and 0.02 pH between 22 and 25ËŽC for LL and HL, respectively see section 4.2.3.). In both cases, no significant impact of temperature between 25 and 28ËŽC can be observed.

Comment 3: In addition, a more rigorous evaluation of temperature effects on aqueous boron fractionation should be performed. For instance, Zeebe (GCA, 2005) and Hönisch et al. (EPSL, 2008) provides guidelines for how this could be done. The actual

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data could then be evaluated within the framework of those theoretical considerations.

Answer: We agree that it would be of interest to better determine the temperature effect on aqueous boron fractionation; however as stated by Zeebe (2007): “Given the range of outcome for α (B3–B4) at 300 K calculated in the current paper, no recommendation will be made regarding α’s temperature dependence, which equally depends on the frequencies/methods chosen.” Moreover these theoretical calculations would need to be tested over a much larger temperature-range and this clearly falls beyond the scope of this manuscript. Here, we appreciate the special note reviewer 3 wrote at the attention of this comment: Reviewer 1’s comment on a temperature effect on alpha: “In the only thorough study of this effect (the Zeebe (2005) paper referred to by reviewer nËŽ1), Zeebe states that: "Given the range of outcome for α (B3–B4) at 300 K calculated in the current paper, no recommendation will be made regarding α’s temperature dependence, which equally depends on the frequencies/methods chosen." i.e. although there is likely to be a temperature effect on alpha, we don’t know it yet (and it may be extremely small over this temperature range). This being the case, adding a temperature effect on alpha is likely to only add confusion and uncertainty.

Comment 4: Similar problems exist for the B/Ca data: The individual data should be provided in Table 3 (now table 4 in the manuscript) and the 1sd uncertainties appear too small: In section 2.4.2 the analytical uncertainty for B/Ca analyses has been reported as 3% at 2sigma. Translating that to the data shown in Table 3 (now table 4 in the manuscript), the uncertainty of each sample should be at least 14 μmol/mol, and that does not yet include any difference between replicates. Comparing this to Figure 5 then suggests that the difference between 400 and 200 μE in B/Ca is not signiïňĄcant. Given that only 3 conditions were analyzed, all replicate data should be shown similar to δ11B data (see above).

Answer: We agree with the reviewer and we apologize for the confusion. We chose to present in Table 4 the standard deviation of δ11B measurements (measured at the end of step 2) based on the different replicates as they appear to be always larger

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than the analytical uncertainties. Therefore, for consistency, a similar process was applied for B/Ca data. However the reviewer is right to mention that in the second case, the analytical SD appears sometimes to be somewhat higher than the one calculated based on the diverse replicates. In order to overcome this problem we recalculated every standard deviation measured at the end of step 2 using a statistical formula allowing the calculation of combined standard deviation (difference between diverse replicates + analytical uncertainties). The new standard deviations (for both δ11B and B/Ca ratios) were corrected in the manuscript and are now presented on table 4 and figures 3 and 5. However for measurements performed at the end of step 3, SD values only represent analytical uncertainties as these values are the results of measurements performed on single nubbins. For clarity this is now clearly stated in caption of Table 4. Also, all replicate data for both δ11B and B/Ca are now shown in Table 4. Nevertheless, in contrary to what stated by the reviewer, this does not change the observed impact of light and temperature on B/Ca ratios as all the statistical tests presented in table 5 were made on the complete set of row data (each replicate considered separately, data now presented in table 4) with the analytical uncertainties for standard deviation.

Comment 5: The discussion of the B/Ca data also shows shortcomings: The introduction presents the basis for this proxy as proposed by Hemming & Hanson (GCA, 1992, not Vengosh et al. 1991!).

Answer: The reference was corrected in the manuscript.

Comment 6: Based on prior studies of B/Ca in marine carbonates, it is clear that borate ion is important for B/Ca, and either carbonate ion or bicarbonate ion. While it should be noted that the control on B/Ca in benthic foraminifers is Delta carbonate ion (i.e. the difference between actual carbonate ion and carbonate ion at saturation in seawater, and not simply carbonate ion as erroneously presented on page 5973 (line 24)), the authors forgot again to consider changes in borate ion under different temperature conditions.

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Answer: On page 5973 line 24 of our manuscript we state: “Recently, Yu and ElderïňĄeld (2007) and Yu et al. (2007) proposed that the B/Ca ratio in foraminiferal calcite can be use as a proxy for seawater [CO32-]”, in their conclusion Yu et Elderfield (2007) state that: “Global benthic B/Ca data show simple linear correlations with deep water Δ[CO32$-$], providing a quantifiable proxy for deep water [CO32$-$] reconstructions”. The fact of using Δ[CO32$-$] as an intermediate step does not change the main application of the B/Ca proxy, which is to reconstruct seawater [CO32$-$]. The statement made in the introduction of our manuscript is perfectly correct. Moreover, one should keep in mind that so far the controlling factors of B/Ca ratios into foraminifera calcite is still subject of debate as reported by Katz et al., (2010): Coretop studies indicate that B/Ca in foraminiferal calcite is strongly influenced by ambient seawater pH, [CO32$-$], or temperature (Yu and others, 2007; Foster, 2008), although the studies disagree on the specific control.”

Finally, the changes in borate ion under different temperature conditions are already largely discussed in section 4.3.2 of our manuscript.

Comment 7: This is difïňĄcult to assess at this point because the carbonate chemistry analyses shown in Table 1 (now table 2) cannot reïňĆect the carbonate chemistry under the respective culture conditions but must have been done at a constant temperature. Because pH decreases with temperature, it is not possible that alkalinity and pH were both constant at 2536 μmol/kg and 8.02 for all temperature conditions. Because alkalinity was determined constant and alkalinity is independent of temperature, pH thus must have differed between the actual culture conditions. A simple estimate using given alkalinity and assuming DIC=2200 μmol/kg, gives a pH of 8.11 at 22âŮęC and 8.02 at 28âŮęC. This difference needs to be considered for all estimates of borate, carbonate and bicarbonate ion, to which the authors should compare their data.

Answer: Here we disagree with the reviewer. An increase in temperature will, by definition, induce an increase in pH as less CO2 can be contained in warmer water, leading to a degassing process of CO2 towards the atmosphere and therewith basification of

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the culturing media with increasing temperature (Zeebe and Wold-Gladrow, 2007). The reviewer assumes a constant DIC (Dissolved Inorganic Carbon) with increasing temperature, which is wrong as DIC will decrease with increasing temperature (as less CO2 will be contained in the medium), while pH will increase and alkalinity will remain constant. The reviewer is right though than none of this is seen in our culturing media. This can be easily explained by the fact that cultures were made in an open system, with the water heater being placed directly within the culture tank, maintaining the temperature of the tank constant. The fast renewal rate of our culturing medium (5 times a day for 30L/aquaria) does not allow the seawater carbonate chemistry to equilibrate to the various temperature and light conditions. This provides a unique opportunity to observe the biological and geochemical response of corals alone to varying temperature and light conditions (deconvolved from the normally co-varying carbonate chemistry of the seawater). For the same reason we can assume that in our study the variation of the skeletal δ11B reflects pH at the site of calcification. This is already stated in section 4. 2: 'Due to the important seawater renewal rate into our culture aquaria (5 times per day), seawater carbonate chemistry remained constant through the experiment, for all conditions (table 2). Therefore, it is assumed that in our study the variation of the skeletal δ11B reflects pH-variations at the site of calcification (Trotter et al., 2011)."

Comment 8: It is also obviously not correct that the measurements were performed "in the culture tanks". Alkalinity and pH samples may have been taken from the culture tanks but measurement must have been done at a different temperature in a vessel outside of the tank. Alkalinity titration in the tank is simply not possible.

Answer: As already described in section II.2. Experimental set-up: "For TA measurements, seawater samples were filtered through 0.45mm membranes, poisoned with Mercury Chloride and stored in a cool and dark place pending analyses (Doe, 1994). TA was determined using a titration system (TIAMO, TITRANDO 888, Metrohm), with a reproducibility of 3 μmol/kg." However we agree with the reviewer that the previous sentence might be confusing, therefore the text was changed as follow: "Salinity

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and pH were measured directly within the culture tanks (Tab. 2, all pHT values in this manuscript are reported in Total scale). pH was measured using a glass combination electrode (Orion 8103SC) calibrated on the Total Scale using Tris/HCl and 2-aminopyridine/HCl Buffer solutions with a salinity of 38 and prepared according to DOE (1994) (accuracy $\pm$ 0.003 pH units). For TA measurements, seawater samples were filtered through 0.45mm membranes, poisoned with mercury chloride and stored in a cool and dark place pending analyses (DOE, 1994). TA was determined using a titration system (TIAMO, TITRANDO 888, Metrohm), with a reproducibility of 3 μmol/kg."

Comment 9: In general, nowhere in the manuscript is any mention of cleaning the coral material before preparation for analysis. Biogenic carbonates, and in particular cultured corals, are loaded with organic matter, which itself can contain signiïňĄcant amounts of boron. Was really no oxidative cleaning protocol applied to remove that organic matter? This is hard to believe but if correct, would jeopardize all analyses. This information needs to be provided to gauge the data quality.

Answer: In contrary to corals from natural environments, cultured corals do not require heavy cleaning protocol as they do not contain any kind of clay or residue that could contaminate the geochemical signature. However, they indeed present organic matter which is a potential source of contamination. In order to remove the organic matter coral aragonite powder was soaked for 12 hours in 30% hydrogen peroxide (Reynaud-Vaganay et al., 1999). The solution was subsequently filtered and rinsed repeatedly with MilliQ water through a membrane filtration (nucleopore polycarbonate with pores 0.45 μm in diameter) chemically compatible with hydrogen peroxide. Each filter was then dried for 2h at 40° prior being handled for boron chemistry. As suggested by the reviewer this is now stated in section II.4. of the manuscript. The consistency in the results between replicates confirms the quality of the analyses.

More speciïňĄc comments: Comment 10 Introduction: There is no experimental indication that "signiïňĄcant concentrations of boric acid" are incorporated in biogenic carbonates. This is later on better described but NMR analyses can only distinguish

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between trigonal and tetrahedral coordination in the carbonate but cannot determine which species was adsorbed in the ïňĄrst place. This has been obvious since Sen et al. (American Mineralogist, 1994) performed phase transformations from aragonite to calcite and observed coordination changes of B in CaCO3 without concomitant changes in d11B.

Answer: We fully agree with the reviewer that the incorporation of boric acid into biogenic carbonates still remains a subject of debate. However, the presence of boric acid in coral aragonite as the product of coordination change (between borate and boric acid) does not represent a problem for boron isotope composition as a proxy for paleo-pH (assuming there is no fractionation during coordination modifications). Only the direct incorporation of boric acid from seawater, would challenge pH reconstruction. This is now clearly stated in the extensive discussion about this topic in the introduction.

Comment 11: Section 2.1 (now section 2.2. in the manuscript)t: A seawater renewal rate of 5 times per day may actually not be that high. I am surprised the temperature should not have varied over a light/dark cycle, and in particular between the two different light treatments. If the temperature was controlled in the external tanks rather than the illuminated culture tanks, as appears to be indicated in Section 2.1 (now section 2.2. in the manuscript), then those temperature conditions may not actually apply to what the corals in their tanks have experienced. This could have signiïňĄcant consequences for the geochemical data and their interpretation and needs to be assessed.

Answer: The heating system was placed within the culture tank not in the external tank. We understand that the description of the pre-heating treatment at 21ËŽC (to adjust the temperature of the water from 55 m depth) prior to flowing into the culture aquaria, might be confusing. The description of the culturing system (and the presence of the temperature controller within the tank) is now clarified in (now) section 2.2. experimental set-up of the manuscript. No doubt remains as to whether the coral experienced the displayed temperatures; the temperature did not vary over a light/dark cycle as it was maintained constant using a heating system coupled with a temperature controller. An

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example of temperature variation within the culture tank during the day/light cycle is shown in the graph enjoined with this letter.

Comment 12: The statistical tests presented in section 3.2 and 3.3. should be performed with individual data measured on the replicates, not their averages.

Answer: As already mentioned in answer to comment 4: all the statistical tests presented in table 4 were already made using all the individual data (now presented in table 4), not the averages.

Comment 13: It should also be acknowledged that the B concentrations measured by Hönisch et al. (2004) were done by isotope dilution and not by MC-ICP-MS. Those measurements are not comparable in precision to ICP-MS analyses and should be considered very carefully. In particular the estimated decrease in B/Ca with pH seems questionable.

Answer: As clearly stated in the manuscript in section "II.4 Geochemical measurements; Boron concentrations: B/Ca concentrations were determined using quadrupole ICP-MS XseriesII (Thermo Fisher Scientific) at the Laboratoire des Sciences du Climat et de l'Environnement (LSCE, France)." Only the Boron isotopes compositions were determined with MC-ICPMS, while the B/Ca concentrations were determined by ICP-QMS. No analytical precision for B concentration is reported by Hönisch et al. (2004). However, Gaillardet and Allègre (1995) report a precision of 2% by isotope dilution using a similar TIMS facility. This is in good agreement with the 3% uncertainties of our study. Our measurements can be considered comparable in precision to boron concentrations measured by isotope dilution (TIMS).

Comment 14: Section 4.1: This section describes the data and then "interprets" them as, e.g., a temperature effect (e.g., line 22-25). Simple data description is not an interpretation, which would include assessments of why patterns change as they do. This entire section could be shortened signiïňĄcantly as it does not ïňĄnd anything new but is only used to conïňĄrm that changes in physiological parameters are consistent

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with earlier studies.

Answer: The word “interpret” line 22-25 was referring to interpretations made by other authors in previously published studies, not to interpret our own data: “Kajiwara et al. (1995) already recorded such a behavior for Acropora pulchra, which was interpreted as an increase in the algal respiration with raising 25 temperature (Karako-Lampert et al., 2004)”. Nevertheless, we agree with the reviewer that this section was too long and we therefore significantly shortened it: see section “VI.1. Metabolic measurements” of the revised version of the manuscript.

Comment 15: Section 4.2.1: It should be noted that Venn et al. (2011) did not perform any temperature experiments, so all the authors refer to in this comparison is the overall offset in pH compared to the site of calciïňĄcation.

Answer: It is true that Venn et al., (2011) did not perform any temperature experiments, but their pH-data are only cited in order to compare the overall increase in pH at the site of calcification vs. seawater, for measurements performed on tropical corals maintained under similar temperature culture condition (25ËŽC). This is now clearly stated in the manuscript: “These increases in pH are in good agreement with the recent study of Venn et al. (2011) on tropical corals using live tissue imaging. They measured a pH increase from 0.2 to 0.5 pH units above ambient seawater under the calicoblastic epithelium of Stylophora pistillata maintained at 25ËŽC.”

Comment 16: The explanation of daily (i.e. day/night) cycles in pH variations at the site of calciïňĄcation and their effect on the integrated geochemical signals should be expanded.

Answer: So far, very few studies report on actual pH measurements at the site of calcification with day/light cycles (all these studies are already cited in section IV.2. 2. Light effect), and no data can be found in the literature about the impact of these daily cycles on boron isotopic signatures. Therefore, although we agree with reviewer that this topic would be of interest for the interpretation of our results, the lack of data/studies

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limits strongly any additional discussion. Moreover one should keep in mind that due to the important seawater renewal rate into our culture aquaria (5 times per day), pH remained constant through the culture experiment during day/night cycles, for all conditions.

Comment 17: Section 4.2.2: The presentation of carbonate chemistry at the site of calciïňĄcation in this section is confusing and seems to suffer from chicken-and-egg problems: Of the three mechanisms described herein, 1 and 3 are essentially the same, as they argue for CO2 uptake by photosynthesis/dinoïňĆagellates. This effect thus should result in higher pH at the site of calciïňĄcation, higher CO32-, and thus improved conditions for calciïňĄcation. If the proton and CO2 concentration were high at the site of calciïňĄcation, CO32- and pH would have to be low and calciïňĄcation would not be favored. This interpretation thus cannot explain the observed data. This problem is extended in Section 4.2.3, where the authors argue once around the observed pH values and once around the calciïňĄcation data, but they cannot bring the two observations in line.

Answer: Both section 4.2.2 and 4.2.3 have been significantly modified and shortened. See answer to comment 1 and 5 from reviewer 3.

Comment 18: Here again it would be interesting to see the true δ11B data spread of individual analyses, to see if the offset is truly signiïňĄcant.

Answer: “True” individual δ11B data per replicates are now presented in table 4, the offset remains significant as shown by the results of the statistical analyses performed on the row data and presented on table 5.

Comment 19: I would also be curious if all geochemical data were measured in the same analytical session, or if an analytical bias could be involved.

Answer: All the boron isotopes data (using MC-ICPMS) were measured in a unique analytical session. As already described in the manuscript: “Instrumental mass frac-

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tionation and drift of the 11B/10B ratio with time was corrected by standard-sample-standard bracketing. Each sample was measured three times successively, with resulting relative standard deviation being systematically in agreement with the external reproducibility of 0.25‰ (2σ) deduced from repeated analyses of boric acid standard NBS-951 and North Atlantic Seawater Standard NASS-V (Louvat et al., 2011; Douville et al., 2010)."

Comment 20: I am also having a hard time putting much faith into the statistical signiïňĄcance of the temperature difference between 22 and 28 âŮę C. Given that only 3 conditions were analyzed and no explanation can be given why this effect should be different between 22-25âŮę C and 25- 28âŮęC, it would be better to consider the entire temperature range rather than only the difference between 22 and 25 âŮęC.

Answer: The results of statistical analyses (performed on row data) are clearly presented on table 5. An explanation to why this effect should be different between 22-25ËŽC and 25-28ËŽC is already extensively discussed in section 4.2.3 temperature effect.

Comment 21: In summary, I am not convinced that this temperature effect is in fact nonlinear. A decrease in aqueous fractionation would be consistent with thermodynamic theory but only if the temperature effect were consistent, not nonlinear. Again, the actual pH seen by the corals in the tanks needs to be taken into account to evaluate the observed differences.

Answer: Statistical results presented in table 5 confirm that the temperature effect is non linear. The actual pH seen by corals in culture tank is presented in table 2.

And Comment 22 Page 5989/line 20: If the enzyme system worked at a constant rate above a certain threshold value, that would not explain higher calciïňĄcation rates, as observed in this study. The argument is therefore ïňĆawed.

Answer: Here we disagree with the reviewer. As already mentioned in the manuscript

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our data suggest that once the pH and hence the carbonate saturation state of the aragonite (Ω) in the site of calcification passes a certain threshold value, the enzyme system responsible for pH increase at the site of calcification works at a constant rate and therewith becomes unreactive to additional temperature enhancement (in the limits of biological sustainability). This can indeed explain the lack of variation in the boron isotopic signature (directly dependent of the pH at the centre of calcification) measured between 25 and 28ËŽC. However, the fact that the pH of the site of calcification remains constant once passing 25ËŽC is non exclusive with the fact that the calcification rate might continue to increase with increasing temperature between 25 and 28ËŽC. Indeed, temperature might impact many other metabolic processes involved in coral calcification. Reynaud-Vaganay et al., (1999) have demonstrated that for Acropora sp. the elevation of temperature stimulates calcification to the optimum temperature of 27ËŽC. Many processes can therefore happen between 25 and 28ËŽC that could explain the observations made in our study, like different enzymes being involved in coral calcification that may react differently to temperature increase. However, at this stage the calcification processes need to be better understood to further hypotheses on this subject.

Comment 23 Section 4.3.1: Considering true analytical uncertainties, the B/Ca data obtained under the two different light conditions are essentially the same.

Answer: See answer to comment 4.

Comment 24 Section 4.3.2/line 14: The light conditions would not have varied between the culture conditions, or were the experiments done outside?

Answer: In line 14 of section 4.3.2 we report about Trotter et al. (2011) experimental conditions. In their study, corals were maintained under controlled cultured conditions (not outside) but: “with irradiance as well as the photoperiod changing according to their seasonal values measured at ca. 20 m depth in the Bay of Villefranche, where the corals had been originally collected.”

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Comment 25: As described above, the pH and temperature dependent changes in borate concentration need to be taken into account for the correct evaluation of borate to carbonate species variations.

Answer: As pointed out by the reviewer we indeed do not calculate the partitioning coefficient of borate in our study. The fast renewal rate of our culturing medium (5 times a day for 30L/aquaria) does not allow the seawater carbonate chemistry to equilibrate to the various temperature and light conditions. Therefore we do not expect the borate concentrations of the culturing media to have adjusted to various light and temperature conditions, but rather be constant. As already explained for boron isotopic signature, this is why we can assume that the geochemical signals measured on our corals present a unique opportunity to observe the response of corals to varying temperature and light conditions alone.

Comment 26: The comment on the debate on temperature effects on B/Ca in planktic foraminifers needs to be either expanded or deleted. Also, references should be provided. Culture experiments with planktic foraminifers have already been performed (Allen et al., 2011, EPSL) and did not reveal a temperature effect on B/Ca.

Answer: The comment on the debate on temperature effects on B/Ca in planktonic foraminifera was expanded as follows: “For planktonic foraminifera for example, while certain studies report a positive relationship between the partition coefficient (KD = [(B/Ca)CaCO3]/[B(OH)4-/HCO3-]sw) and temperature (Globorotalia inflata, Globigerina bulloides from coretop samples, and Globigerinoides ruber from downcore, Tripati et al., 2009; Yu et al., 2007), others observed a negative KD-T ËŽC relationship (Globigerinoides sacculifer, G. ruber, and Neogloboquadrina dutertrei from coretop; Foster, 2008). Similarly, when B/Ca ratios is observed to increase with temperature in Globorotalia inflata (Yu et al., 2007), no temperature influence can be seen on Neogloboquadrina pachyderma (sinistral) (Hendry et al., 2009). Recently, culture experiments made by Allen et al., (2011) on the planktonic foraminifera Orbulina universa, did not indicate any temperature effect on B/Ca ratios (B/Ca values measured on shells grown

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between 17.7 and 26.5 °C agree within error with a slope statistically indistinguishable from zero). Considering the varying responses of foraminifera shell B/Ca ratios to temperature, Allen et al. (2011) recommend applying empirical, species-specific temperature calibrations for paleo-reconstructions. These observations suggest that the controlling factors and processes driving boron transport to the calcification site and its incorporation into marine calcium carbonates have not been adequately identified yet. Additional species-specific experiments combining both cultured and naturally-grown samples are necessary to improve our understanding and therewith the use of B/Ca as an environmental proxy."

Comment 27 Section 4.3.3: Here again the effect of variable culture water pH and temperature on B/Ca need to be removed before comparison with other data is possible.

Answer: See answer comment 25

Comment 28 Section 4.3.4: The presentation of the recovery data omits important data. In Figure 4 the authors must have assumed that corals record pH at the site of calciïňĄcation after Klochko and thus placed their data onto the d11B of borate curve. However, there is some inconsistency in this exercise because following that line of argument, all 5 data should fall on the solid line, and not some on the solid and some on the dashed line.

Answer: The reviewer is right and figure 4 was corrected. By mistake, the pKB values correlated with the temperature condition of step 2 were considered (pKB values of 8.62 and 8.58 were used for pH reconstruction based on nubbins from the 22 and 25 treatments during step 2, respectively) rather than only taking into account the pKB value for 25ËŽC as all nubbins during step 3 were placed in the same temperature condition. All values now fall on the solid line. Data were corrected in figure 4 and table 4.

Comment 29: A crossplot of this pH estimate with the B/Ca data (using data provided in Table 3 (now table 4 in the manuscript)) shows that there is no signiïňĄcant correlation

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between the two parameters. This is an interesting piece of information, as it either reïňĆects on the assumption that pH (or some related carbonate parameter) is the unifying parameter between both proxies, or on the data quality. Again, individual analyses data should be provided for all data collected in this study. There is some agreement between B/Ca and d11B in that the data are overall lower compared to phase 2 data but the crossplot does not show much consistent behaviour beyond this.

Answer: We agree that B/Ca ratios and δ11B show slightly different response to increasing temperature, and therefore plot slightly different trend versus reconstructed pH. At this stage, however, the controlling factors and processes driving boron concentration into coral aragonite have not yet been adequately identified (see section IV.3. 2. Temperature effect). Moreover our data remain too limited (more experimental condition would be required) to draw significant conclusions about what parameter might be unifying both proxies. All individual analyses are now provided on table 4. B/Ca ratios and δ11B measured at the end of step 3 show indeed inconsistent responses to environmental parameters, with nevertheless, much lower values than the one measured at the end of step 2 for both B/Ca and δ11B. As already mentioned in section IV.3. 4. B/Ca ratios after recovery experiment this tends to confirm that the mechanical stress applied to the coral between step 2 and step 3, led to a perturbation of the pH enhancement process at the site of calcification.

Comment 30: In summary, the data set is interesting but shortcomings in the culture procedures; cleaning, data presentation and evaluation need to be addressed. The data evaluation should be much more rigorous and include thermodynamic considerations in addition to the simple comparisons with environmental parameters.

Answer: Every potential shortcomings pointed out by the reviewer (culture procedures, cleaning, data presentation and evaluation) have been answered. Thermodynamic considerations are extensively examined and argued within the discussion section of this manuscript (e.g. section 4.2.3, 4.2.4, 4.3.1.). We are thankful for the reviewer's constructive comments.

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Interactive comment on Biogeosciences Discuss., 9, 5969, 2012.

Temperature *vs* time of the culturing medium in the 25°C tank.

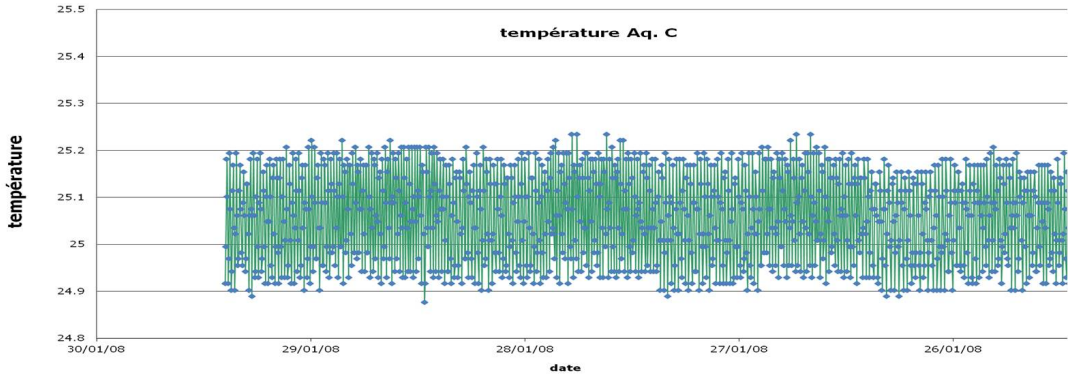

Fig. 1.

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