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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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Answer to anonymous referee nËŽ3 Dissard et al. report ïňĄndings from a thorough study of the effects of temperature and light intensity on boron isotopes (d11B) and boron/calcium ratios in the coral Acropora. This work is topical, the experiments are thorough, and the data is interesting, and I recommend publication after some minor revisions.

Answer: We greatly appreciate the positive feedback we received from reviewer nËŽ3. The suggested changes were taken into account as follows: (each comment was addressed separately)

General comments

Comment 1 Explanation of light effect on d11B.

One of the most interesting ïňĄndings of the study is the fact that with increasing light intensity, d11B decreases. This is unexpected, as increasing light intensity increases photosynthetic rate, which might be expected to increase CO2 uptake, and thus increase pH in the coral epithelium and microenvironment; this would be expected to increase d11B (and B/Ca) in the coral skeleton, rather than cause the decrease that is observed. Furthermore under high light intensity, calciïňĄcation rate increases (an effect known as light-enhanced calciïňĄcation - LEC), which has often been attributed to an increase in pH due to increased photosynthetic rate. However what the authors show is that the opposite seems to take place: high light does increase photosynthesis and does enhance calciïňĄcation, but that pH seems to be lower. The authors' description of possible reasons for this is currently very confusing. I think this stems, in part, from taking the summary given by Moya et al. (2006) as a starting point for this discussion (5985, 25 - 5986, 7). I don't think this summary is clear, even having read the original references in question. Point 2 is too vague, as several carbon concentrating mechanisms have been suggested, and the main ïňĄnding of the study of Furla et al. 1998 is similar to point 1: that enhanced light causes an increase in epithelial pH (with the addition that this may have relationships with enhanced ion channel transport). Point 3 describes a feedback between calciïňĄcation and photosynthesis, with calciïňĄcation removing alkalinity relative to DIC in a 2:1 ratio, and thus shifting the carbonate system towards CO2, which may then be used for photosynthesis. This removal of CO2 (DIC) then increases pH (and CO32-) again, and thus may promote calciïňĄcation. However as admitted by the authors (5987, 2): "this seems more a consequence than a cause" of light enhanced calciïňĄcation. More importantly, I don't agree with the authors that this process will lead to a net lowering of pH in the calcifying environment. The calciïňĄcation step will lower pH, but this is the case in all these examples. The step linking in photosynthesis (i.e. that the CO2 produced during cal-

ciïňĄcation may be reabsorbed during photosynthesis), will cause an increase in pH, and is thus just the same as point 1. The authors don't need to solve light enhanced calciïňĄcation in one fell swoop. As none of the explanations really seem to work, they should just clearly describe this and leave it as an interesting result, deserving future study. Alternatively, if I've made a mistake in the logic above, and the authors think that mechanism 3 really does work to cause a net decrease in pH in the calcifying environment, they need to show this much more clearly, ideally with some modelling, or at least graphically.

Answer: We agree with the reviewer and we thank him for this very valuable comment. The fact that increasing light intensity induces decrease d11B is indeed one of the most interesting finding of our study. We agree that the description of possible reasons for this based on the summary given by Moya et al., (2006) might be a bit confusing and indeed point 1 and 3 can become circular with regards to pH variation at the site of calcification. Therefore for clarity, we decided to rewrite and shorten this section. The summary given by Moya et al., 2006 has been removed and simplified by the two most admitted hypothesis involving modifications of carbonate chemistry inside the coelenterons with LEC processes. (Please see new section: IV.2. 2. Light effect of the revised manuscript).

Comment 2: Description of culturing This work will be of interest to the isotope geochemistry and paleoproxy community. As such, I think the description of the culturing could be made slightly clearer. I'd suggest making a table or schematic that describes the 3 steps within the culturing process.

Answer: We agree with the reviewer and for clarity a table describing the 3 steps of the culturing process was added to the manuscript, it is now referred in the manuscript as table 1. Also the material and method section was reorganised. Section II.1 and II.2 were inverted. Section II.1, now called experimental protocol, describes the three different culturing steps and refers to table 1. Subsequently, section II.2 describes in more details the culturing set-up. The description of the metabolic measurements is

now entirely contained in section II.3 (see answer comment below).

Comment 3: I was also confused about the pieces of coral used for metabolic measurements – were they kept in the same culture tank as their associated nubbin, and just brought out for the metabolic measurements? And do coral pieces grown on wires behave the same as those grown on slides?

Answer: Yes, the different pieces of coral used for metabolic measurements were kept in the same culture tank than their associated nubbin, and were indeed only brought out for metabolic measurements. This is now clearly stated in section II.3. Metabolic measurements: "Nubbins glued onto slides could not fit in the incubating chamber, therefore, all metabolic measurements such as respiration, photosynthesis, and calcification rate were conducted simultaneously on small fragments, from the same parent colony, hung on nylon wire (Al-Moghrabi et al., 1993) and cultured in the same culture tank as their associated nubbin (Tab. 3)." So far, no studies report on metabolic behaviour comparison between nubbins of similar size glued onto slides vs. nubbins hung on nylon wire. However, over 20 years of experience of culturing corals under both forms at the Scientific Centre of Monaco, physiological responses always happened to be extremely similar. It can be safely assumed, that if existing, differences must remain really small and should not significantly alter the estimation of our metabolic measurements, Comment 4: Finally please annotate Fig. 1 to show the new growth of aragonite in culture, and here and in Section 2.2 describe how skeleton grown in step 1 was distinguished from skeleton grown in step 2.

Answer: As suggested by the reviewer the newly grown aragonite is now annotated in Fig. 1. At the end of step 1, the skeleton was only present until the limit of the glue, but never on the slide. Nubbins were then transferred into the experiment (step 2) where they grew onto the slide. Only aragonite precipitated on the slide was sampled at the end of step 2 allowing the collection of newly formed aragonite precipitated under the targeted culture conditions. This is now clearly stated in section 2.2. of the manuscript.

Comment 5: Shorten description of other work. I think there are several places where this manuscript loses ïňĆow by too much detailed description of previous studies, with little reference to the new work in this paper. Shortening these sections (for instance 5988, 18 - 5989, 9) would make the paper more focussed and much more readable.

Answer: We agree with the reviewer and these sections were shortened as follow: “A mechanism involving the removal of protons generated during calcification via Ca2+ATPase activity was proposed to be responsible for the observed pH increase (and therewith increase in [CO32-]) and Ca2+ concentration of coral site of calcification (Cohen and McConnaughey, 2003; McConnaughey and Whelan 1997, Al-Horani et al., 2003). More recently, Herfort et al. (2008) reported that additions of NaHCO3 to synthetic seawater proportionally increased the calcification rate of Acropora sp. This indicates that the concentrations of carbonate species ([HCO3-] and/or [CO32-]) rather than calcium, are the limiting factor of coral calcium carbonate precipitation. Carbonate system equilibria are such that CO2 is more soluble in cold water. Hence, an increase in temperature leads to a decrease in [CO2(aq)] and a subsequent decrease in [HCO3-] and increase in [CO32-].”

Reviewer 1’s comment on a temperature effect on alpha Comment 6: I disagree with reviewer 1’s comment that: "rigorous evaluation of temperature effects on aqueous boron frac- tionation should be performed. For instance. Zeebe (GCA, 2005) and Hönisch et al. (EPSL, 2008) provide guidelines for how this could be done". In the only thorough study of this effect (the Zeebe (2005) paper referred to by this reviewer), Zeebe states that: "Given the range of outcome for αB3–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.

Answer: We agree we reviewer nËŽ3, and we thank him for taking the time to write this

extra comment. This point is now extensively discussed in comment nËŽ3 of reviewer nËŽ1.

Comment 7: The reviewer is, however, right that changes in pKb with temperature should be evaluated.

Answer: We agree with the reviewer on this point as well. As already stated in answer to comment nËŽ2 of reviewer nËŽ1, changes in pKb with varying temperatures were already considered in our pH reconstructions. This is now clearly stated in the manuscript in section 4.2.1.

SpeciïňĄc comments and technical corrections

5971, 9: mention in the abstract the ïňĄnding that increased light causes a decrease in d11B, the opposite to what is expected in most models of light-enhanced calciïňĄcation. and comment 5971, 11: it would be helpful to give example real world conditions or environments that this change in light intensity represents, i.e. "equivalent to the summer vs. winter light intensities in the natural environment of these corals".

Answer: The following sentence was added to the abstract: ‘Changes in light intensities from 200 to 400 μmol photon m-2 s-1 induces a decrease in pH of the site of calcification of about 0.03, 0.04 and 0.03 pH-units at 22, 25 and 28 ËŽC, respectively. These light variations, chosen to mimic average annual variations in natural environments where Acropora sp. can be found, only biased pH reconstructions by about 0.05 units.”

5971, 12: replace "between 22 and 25 C" with "with an increase from 22 to 25 C" and "enhancement" with "an increase" for clarity.

Answer: The sentence was corrected as follow: ‘For both light conditions, a significant impact of temperature on δ11B can be observed between 22 and 25 ËŽC corresponding to an increase of about 0.02 pH-units, while no further δ11B increase can be observed from 25 to 28ËŽC.”

5971, 16: replace "conïňĄrming" with "consistent with" as B/Ca and d11B may have different controls.

Answer: The sentence was corrected as suggested by the reviewer.

5971, 19: replace "ions" with "ion"

Answer: The word “ions” was replaced by “ion”

5971, 25: replace "(actual)" with the year this value applies to.

Answer: The word “actual” was replaced by the “2010” and therewith the value ‘380ppmV” was corrected to “390ppmV”.

5972, 3: insert "past" before "seawater"

Answer: The word past was added as suggested by the reviewer.

5973, 1: as the authors discuss in this paragraph, these NMR measurements record trigonally coordinated boron in a crystal, which is not necessarily the same as boric acid and doesn’t necessarily imply boric acid incorporation (see Klochko et al. 2009 Figure 9). So please replace "boric acid" at line one with "trigonally coordinated boron", and in line 3 say something like "if this reïňĆected incorporation of boric acid in this proportion..."

Answer: The text was corrected as follow: “This assumption was recently raised by two NMR studies, which measured proportions of trigonally coordinated boron in coral aragonite varying from 12 to 48 % (Klochko et al., 2009; Rollion-Bard et al., 2011). However, NMR technique cannot distinguish between boric acid directly incorporated from solution or derived from borate ion during adsorption and incorporation into boron-carbonate. Recently, Tossell et al. (2005) and Klochko et al. (2009) have pointed out the possibility of a “chemosorption stage” where B(OH)CO3- isomers may form on the surface before breaking down into either BO3 or BO4 in natural carbonates. In turn, this may result in internal boron isotope redistribution which would allow both BO3

and BO4 to be incorporated while preserving the internal (site of calcification) δ11B isotopic composition. In addition, because the incorporation of seawater boric acid would shift the boron isotopic composition of coral skeletons to considerably higher values (McCulloch et al., 2012), it appears unlikely that the trigonal B(OH)3- species detected in calcite and aragonite is directly derived from seawater.”

5973, 17: refer to Juillet-Leclerc references as submitted, rather than giving it a year, which implies it is already published.

Answer: The reference was corrected in the manuscript.

5975, 1: indicate new skeleton growth in Figure 1.

Answer: The new skeleton growth is now indicated on figure 1.

5976, 8: replace "steps" with "step"

Answer: The word step was corrected in the manuscript.

5976, 9: how many nubbins per experiment?

Answer: The number of nubbins and therewith or true replicates is now stated in this section (II.2. Experimental protocol): “The nubbins were subsequently randomly distributed within six tanks under six different conditions (200, 22; 200, 25; 200, 28; 400, 22; 400, 25 and 400, 28), where the first number indicates the light intensity (μmol photons m-2 s-1) and the second one the temperature (°C) (two nubbins per tank, except for condition 400, 28, where three nubbins maintained in culture).”

5976, 16: how was skeleton grown in step 1 distinguished from that grown in step 2?

Answer: Coral are placed in step 2 before starting to precipitate aragonite on the slide (only on the glue). Vice versa only the skeleton formed on the slide is removed and analysed at the end of step 2. This way, we ensure that skeleton analysed at the end of step 2 has been formed under the targeted controlled environmental conditions.

5976, 24: give a reference for the respirometry technique, and replace "consists in" with "consists of"

Answer: A reference was given for the respirometry technique (Griffith et al., 1987) and “consists in” was replaced by “consists of” as suggested by the reviewer.

5977, 6: what is LT?

Answer: LT stands for Local Time, it is now written fully in the manuscript.

5977, 8: what is "nitrogen-bulled"? Grammar here doesn’t seem quite right.

Answer: We meant to write “nitrogen-bubbled”, it is now corrected.

5977, 12: replace "weighted" with "weighed"

Answer: The word was corrected.

5977, 13: replace "were" with "was" and provide a reference for this formula (or if original give rationale for its use).

Answer: A reference was provided for this formula: Reynaud et al., (2002).

5977, 19: what type of replicates are these - fully separate samples from different nubbins, separate samples from the same nubbin, replicate analyses of the same coral sample, replicate analysis of the same dissolved solution etc.?

Answer: The replicates are fully separate samples from different nubbins incubated in the same aquaria, 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.”

5977, 23: what sample size was analysed?

Answer: The sample size analysed was 55mg of powdered aragonite, this is now

clearly stated in the manuscript in section 2.4: Boron isotope measurements: “The total quantity of material considered was 55mg of powdered aragonite per samples.”

5978, 5: does this 0.5 permil refer to at the size of the blank expressed as a fraction of the total sample, or to the effect of the blank on isotope composition? Make this clear.

Answer: This 0.5 permil refer to the effect of the blank on isotope composition, this is now clearly stated: “The direct injection technique allowed a strong reduction of the analytical blank contribution on isotope composition (lower than 0.5‰ of the sample signal for each isotope).”

5978, 12: replace "than" with "to"

Answer: “Than” was replaced with “to”.

5981, 1: give references for these values. Bt has been remeasured by Lee et al. 2010 (432.6 umol/kg), more precisely than this measurement by Uppstrom 1974. Ideally the value of Lee et al. should be used. However as the value of Uppstrom is still given in the best practices guides by EPOCA and Dickson 2007, I suppose it is OK to use the Uppstrom value.

Answer: The value used in the manuscript (416 μmol/kg) is from DOE (1994). We used this value to help comparison with previously published studies as, to date, this is the most commonly recognized and used value (e.g. Klochko et al, 2006). Reference was added to the manuscript.

5984, 17: replace "important" with "high" or some such.

Answer: The word “Important” was replaced with “high”.

5984, 20: this is not necessarily the case - kinetic effects or some other fractionation of the two molecular species could occur during incorporation of boron into carbonate. This needs to be stated clearly.

Answer: Here we agree with the reviewer, however even if an “in between” process

occurs during the incorporation of boron into the carbonate, the final signature will still reflect what the coral aragonite records from the pH of the site of calcification (rather than the culturing media pH). The “in between” processes should be considered as “vital effects” and they cannot be discarded from the isotopic signature.

5985, 12: replace "up-regulation" with "pH up-regulation"

Answer: The correction was applied as suggested by the reviewer.

5985, 15: see discussion of this section in major comments above.

Answer: See answer to major comment above.

5988, 2: replace "paleo-pH reconstructions are still..." with "paleo-pH reconstructions from corals are still...", as reconstructions from other species may be better or worse than this.

Answer: The sentence was corrected as suggested by the reviewer.

5988, 18 - 5989, 9: much of this section could be cut.

Answer: See answer comment 5.

5989, 5: cut "By deïňĄnition". Could replace with "Carbonate system equilibria are such that CO2 is more soluble in cold water" or some such.

Answer: The sentence was corrected as suggested by the reviewer.

5989, 12: I don’t understand what is meant by "carbonate availabilities" - please be more clear.

Answer: With “carbonate availabilities” we refer to what is already stated two sentences before “ Hence, an increase in temperature leads to a decrease in [CO2(aq)] and a subsequent decrease in [HCO3-] and increase in [CO32-] (for instance, under similar conditions an increase in temperature from 5 ËŽC to 25 ËŽC leads to a [CO32-] increase of 90 %).”

5990, 3: change section title to "Comparison of the impact of light vs. temperature"

Answer: The title was corrected as suggested by the reviewer.

5990, 3: Currently not really sure what this section adds. Could be improved by clearly stating at the start of this section that despite seeing an increase in calciïňĄcation under both increased temperature and increased light intensity, these conditions have the opposite effect on d11B.

Answer: To accommodate this comment this section was significantly shortened and is now the last point of section IV.2. 3. Temperature effect: “It should be noted here, that despite seeing in our study an increase in calciïňĄcation rate under both increased temperature and increased light intensity, these conditions have the opposite effect on boron isotopic signatures. However, one should keep in mind that these two environmental parameters might influence calcification on many different levels. Temperature, for example, is known to impact metabolic processes (e.g. enzyme activity), which in turn might account for the observed differences in pH regulation. At this stage calcification mechanisms need to be better understood to fully quantify light and temperature effect on δ11B-pH proxy.”

5990, 10: again, this mechanism doesn’t really work.

Answer: This sentence was removed from the manuscript.

5991, 5: B(OH)4- doesn’t become the dominant species at these pHs and pKbs - it just increases in abundance. B(OH)3 is still the more abundant species.

Answer: The sentence was modified as follow: “An increase in pH of the calcification site increase B(OH)4 concentrations (Hershey et al., 1986; Hemming and Hanson 1992), and the boron concentration in the coral is proportional to the boron activity in the precipitating solution (Kitano et al., 1978; Vengosh et al., 1991; Hemming and Hanson 1992).”

5991, 18: replace "conïňĄrm" with "are consistent with", as B/Ca and d11B may have

different controls.

Answer: The sentence was corrected as suggested by the reviewer.

5994, 22: state the interesting result that increased light intensity results in lower d11B.

Answer: The sentence: “Changes in light intensities from 200 to 400 μmol photon m-2 s-1 induces a decrease in pH of the site of calcification of about 0.03, 0.04 and 0.03 pH-units at 22, 25 and 28 ËŽC, respectively.” was added to the conclusion.

5995, 4: again replace "conïňĄrming" with "consistent with"

Answer: The sentence was corrected as suggested by the reviewer.

Table 2 (now tab. 3): Give units for CalciïňĄcation Rate, and maybe use "Calcn rate" as abbreviation, rather than "Ca rate".

Answer: Calcification rate unit is now given in tab 3, and for clarity “Ca rate” was corrected by “calcification rate”.

Fig. 4: replace "Oranges crosses" with "Orange crosses".

Answer: The word was corrected.

Fig. 5: again, reference to Juillet-Leclerc should be submitted, not 2012.

Answer: The reference was corrected.

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