

## ***Interactive comment on “Enhanced pH up-regulation enables the cold-water coral *Lophelia pertusa* to sustain growth in aragonite undersaturated conditions” by M. Wall et al.***

**Anonymous Referee #1**

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Wall et al. studied skeletal morphology, crystal orientation and boron isotopic composition of the cold-water coral *Lophelia pertusa*, comparing samples grown in their natural environment with those grown in laboratory culture under high pCO<sub>2</sub> and undersaturated conditions with respect to aragonite. This is a potentially interesting study that may shed some light on the mechanism by which this species manages to calcify in undersaturated conditions. However, the study lacks rigor in several aspects, including the description and/or design of the culture experiment, quantitative analysis of results and rigorous discussion of the new findings in comparison to earlier publications.

First of all, it should be mentioned in the abstract that this study compares natural and cultured samples. “Acclimation” to low pH should also be used with caution, because

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we do not know whether the corals generally elevate their calcifying fluid pH far beyond external pH or whether they specifically acclimate to low pH conditions. The d11B data in *Lophelia* and *Desmophyllum* are the highest observed in any marine carbonate to date, and certainly suggest that such pH elevation is likely a general pattern. Whether the pH elevation at low ambient pH is greater than at moderate ambient pH is a different question, more on that below.

Experimentally, by comparing natural and cultured samples, it should be evaluated whether the culture conditions create the observed morphological and geochemical differences. For instance, food supply could be different in culture compared to the natural environment, which would affect respiration rates and organismal CO<sub>2</sub> production; a control experiment under simulated natural conditions (minus the pressure effect) would be valuable. When then comparing skeletal morphology under the different experimental treatments, branches of similar diameter should be studied, so that any size effect can be excluded. Figures 2-5 describe morphology and boron isotope data from old, new, young and side branches. What is the difference between such branches, do some grow more than others, and which ones were used for the geochemical analyses? How long were the corals kept in culture for? Was the duration the same for all treatments? This information may be provided in Form and Riebesell (2012), but should be described briefly here.

The data shown in Figure 1 show supposedly no systematic difference between the treatments (page 6765), but to me it looks as if the corals grown under high pH grow thicker skeletons. This is corroborated by progressively smaller scale bar sizes used for Figures 1e,f and g,h. Data of this kind (both calcite and organic carbon layers) should not only be shown as images, but quantitative measurements need to be shown in a graph. In addition, how many specimens have been evaluated for this comparison? Judging from supplementary Table S2, five natural specimens may have been compared to two cultured specimens. Is this sufficient? What is the individual variability between specimens? The d11B data seem to suggest that individual variability is large,

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and authors appear to agree with this. However, because the analytical uncertainty of SIMS is very large, what can we really expect to infer from these samples?

As a very basic approach, I would have expected to find a prediction of the  $\delta^{11}\text{B}$  difference between natural samples and the high  $\text{pCO}_2$  treatment. Following the boron isotope fractionation factor of Klochko et al., the  $\text{pKB}$  values and environmental and experimental conditions, one can predict  $\delta^{11}\text{B}$  of the coral skeleton in the high  $\text{pCO}_2$  treatments should be  $\sim 1.8\text{‰}$  lower compared to the natural sample. Of course, this depends on how accurately we know the natural conditions, and it also depends on appropriate conversion between pH scales. The manuscript uses the seawater scale (line 1, page 6760), the free scale (Table S1), and probably also the total scale, because that is the pH scale underlying the study of Dickson 1990 (line 15, page 6763). There is at least a 0.1 pH unit difference between the free and seawater scale, the total scale typically differs from the seawater scale by  $\sim 0.01$  units. It should be clarified whether different scales have been used at the collection site and in laboratory culture, and if so, if the data have been converted appropriately.

Given that the uncertainty of the SIMS analyses ranges from 1.4-2.69‰ (Table S2), do the authors really expect to see a significant signal with such a small sample collection? Furthermore, the data collected here are much lower than those published by Blamart et al. (2007) on the same species, but both studies used the SIMS technique. Other than saying that these new data make more sense than the previous study, the authors do not discuss the reasons why they are so different. Is this just a standardization issue or is there more behind it?

Whatever the reason for the analytical difference, Rollion-Bard et al. (2011) used the data of Blamart et al. (2007) to compare their NMR data to. Because Rollion-Bard et al. assumed Blamart's data accurate, they combined them with their estimated contribution of boric acid over borate ion incorporation. It is not surprising that the same approach does not hold for these new data, which are more than 10‰ lower than Blamart's data. Furthermore, the authors should note that the entire NMR debate is

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fundamentally flawed because NMR cannot distinguish between boric acid and borate adsorption, it can only identify whether boron in the crystal lattice is in trigonal or tetrahedral coordination. It has already been shown by Sen et al. (1994) that Boron changes its coordination in the crystal in response to phase transformation from calcite to aragonite. It has been shown in many studies (e.g. Klochko et al., 2009, Allen et al. 2011) that the boron isotopic composition predicted from boron coordination in marine carbonates should be much higher than measured by various analytical techniques (TIMS, MC-ICP-MS, and now also the new SIMS data presented by Wall et al). This continued comparison of boron coordination and isotopic composition is simply not useful and should be abandoned. Furthermore, Figure S5 is of poor quality and if the authors want to show it, they should calculate the lines themselves and prepare a new figure, instead of superimposing their data on the published (copied) Figure.

With regard to the pH up-regulation argument, the authors should bear in mind that inorganic calcite (Sanyal et al., 2000) shows a similar "up-regulation" at low pH compared to aqueous borate as all other marine carbonates calibrated to date. This fact is categorically dismissed in boron isotope studies that aim to infer pH regulation on corals. It is correct that Kühl et al. (1995) and Al-Horani et al. (2003) found pH variations at the site of calcification that are related to symbiont photosynthesis, but deep-water corals do not harbor photosymbionts, the argument made on page 6759 is therefore somewhat irrelevant. Venn et al. (2011, 2013) and Holcomb et al. (2014) found clear evidence that *S. pistillata* upregulates calcifying fluid pH more at low ambient pH compared to high ambient pH treatments. It is therefore possible that the high  $\delta^{11}\text{B}$  recorded by *Lophelia* and *Desmophyllum* at low ambient pH may indicate active pH upregulation. However, so far we have only evidence from the one species (*S. pistillata*), grown in the same laboratory. Given that inorganic  $\text{CaCO}_3$  (Sanyal et al. 2000) records the same "vital effect" in  $\delta^{11}\text{B}$  as corals, it is equally possible that we are missing an aspect in the understanding of the boron isotope proxy that creates this deviation at low pH, and that the  $\delta^{11}\text{B}$ -deviation does not have anything to do with greater pH up-regulation at lower ambient pH. The inorganic precipitation experiments

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should be repeated but until we have contrasting evidence for inorganic  $\text{CaCO}_3$ , using  $\delta^{11}\text{B}$  to argue for pH up-regulation is more than questionable. Consequently, the discussion of this topic should be phrased a little more carefully. The authors may also want to consider how dissolved boron reaches the site of calcification in *Lophelia* (and *Desmophyllum*). Given that these species live in unusual conditions, they may have developed ion pumping strategies that allow elevated uptake of boric acid (as the uncharged species) over borate ion. A skewed uptake ratio of boric acid over borate could increase the recorded  $\delta^{11}\text{B}$  just as much as up-regulation of the calcifying fluid pH. This comparison demonstrates that physiological interpretation of proxies with incompletely understood systematics may be misleading. Application of pH-sensitive dyes (similar to Venn and Holcomb's studies) would be a very useful comparison to verify the boron isotope observation in *Lophelia*. This goes obviously beyond the scope of the current study, but unless it is done, the discussion should be presented with caution.

Some minor issues that would benefit from greater detail:

What is meant by "with precautions concerning its use for deep water corals" (page 6763)?

Page 6768: what are the important consequences for the boron isotope proxy in *Lophelia*?

The discussion paragraph starting on line 4, page 6768 should be introduced. It is not clear where it wants to go, and the authors have not studied food supply in their experiments, so this entire discussion is somewhat speculative and poorly corroborated. Of course, the discussion of food supply also begs the question whether the natural samples presented in this study are really a suitable reference for the high  $\text{pCO}_2$  experimental group?

Page 6769: please discuss why one should be worried about changes in the organic layers when the  $\text{CaCO}_3$  skeleton is as thick or thicker under undersaturated conditions

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compared to saturated conditions?

Table S1 mixes commas and periods, please choose one for all

Table S2: The transect numbers are not easily identifiable in main text figures, and there are fewer figures in the main text than transect numbers. These values should be easily identified within the table, e.g. by adding another line identifying natural from cultured samples, and ordering them accordingly.

Figure S2, second caption paragraph: Referring in the figure caption to the same figure seems odd. This caption is somewhat unclear.

Most figures: Don't use the differential operator symbol instead of the delta symbol.

Figure S4: The red asterisk can barely be seen, use a different color, e.g. blue.

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Interactive comment on Biogeosciences Discuss., 12, 6757, 2015.

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