Authors Comment:

We would like to thank the two Referees and Rodolfo-Metalpa for their constructive review and comments and would like to address and clarify their comments, concerns and questions.

Referees comments:

Anonymous Referee: First of all, it should be mentioned in the abstract that this study compares natural and cultured samples.

Answer: We agree with the referee that the origin and conditions during which the coral was precipitating the skeleton is important and we will add this information into the abstract: “We compared the isotopic composition and structure formed in their natural environment to material grown in culture at lower pH conditions.

Anonymous Referee: “Acclimation” to low pH should also be used with caution, because 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 6^14B 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.

Answer: Here we would like to clarify that the term acclimation was used in the abstract in reference to the previous publication by Form and Riebesell (2011, GCB: Acclimation to ocean acidification during long-term CO2 exposure in the cold-water coral Lophelia pertusa). They showed that these corals acclimatised to low pH during culturing by maintaining, even elevating calcification rates. However, we will be careful with the use of the term acclimation and have consequently modified the text to reflect this.

Anonymous Referee: 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 CO2 production; a control experiment under simulated natural conditions (minus the pressure effect) would be valuable.

Answer: As the reviewers correctly states, difference in the environment such as food availability could impact not just the growth and the physiology but also potentially the material properties, though we are not aware of any study showing such a link. We agree that a control experiment under simulated natural conditions in particular in respect to in-situ food regime would be valuable but, to date, does not exist. Only one study by Rodolfo-Metalpa et al. 2015 evaluated in-situ growth in several cold-water coral species and suggests food limitation potentially accounts for lower in-situ growth rates compared to rather “well-fed” cultured corals which is in agreement with findings based on other model organisms. We are confident that we can draw conclusions from boron isotopes that the corals sustained their calcification pH within the errors of the methodology, and suggest this was made possible by sufficient energy through food uptake.

Anonymous Referee: 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.

Answer: We agree with the need for more details on the experiment and sample selection and will provide the information in the revised manuscript. The duration of changed pCO2 conditions was the
same for all treatments and lasted 6 month. Only polyps grown at the distal ends of the colony were used for geochemical analysis and structural analysis. Form anaylsed skeleton morphology of *L. pertusa* for this culture study and found no relationship between linear extension rate and calyx diameter ($r^2 = 0.04$, n = 60). Thus, we are confident that polyp diameter will not affect our results. All this information is added to the revised version of the manuscript.

**Anonymous Referee:** 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.

Answer: Fig 1 illustrated cuts below and above the stainline and compared within specimen. Within treatment the same scale bar was used. We agree that a numerical treatment was needed to ensure our readers that growth is not changing and added quantitative measurements to underline our results. Since diameter had no influence on polyp extension (see above), we are confident that we can analyse different parts of the specimen and assess the thickness of the structures. Independent of size prior to the experiments, none of the treatments show significant changes in wall morphology. Using synchrotron tomography allowed us to assess a number of hypothetical sections and we found that the skeleton below staining line can be both thinner and thicker compared to the new growth. Our findings indicate that once a certain diameter and morphology is achieved, the new growth of the polyp does not change under changed $p$CO$_2$ conditions resulting in the lack of trends in wall thickness.

In the Raman map from the high $p$CO$_2$ treatment, we observed a change in the organic matrix layers within the wall structure. This was only an observation, which we believe is important to note, however, it is not quantifiable as such given our current methodologies.

Future studies shall go into more detail whether the amount of organic material/composition of the organic material (as indicated by genetic observation Moya et al. 2006 and observed by a lab study of Tambutte et al. 2015) as well its arrangement will be affected in the future. In the manuscript we will discuss it more carefully to clarify our observation.

**Anonymous Referee:** 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, C2114 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?

Answer: We have clarified the number of specimen we have analysed and the effort we have made to ensure that our data is meaningful. Firstly, we show that our data are reproducible if we measure the same polyp in cross section at different positions. Secondly, we have measured two branches of the same organism and do get the same $^{6}$Li values and hence pH reconstruction. Hence we are confident that the values for individual specimens are sound. Thirdly, the reconstructions of the natural growth from two specimen shows that we get similar values within error for the natural growth.

For the two individuals analysed we do not see differences between natural growth and experimental material grown under low seawater pH. This points towards the corals ability to sustain internal pH and hence saturation. We do agree with the reviewer that it would be desirable to grow a large number of individuals and also, see above, at different pH conditions but the low growth of the individuals is a larger hindrance to this. With regards to the error of the analysis, the SE of our analysis is significantly higher than a TIMS or MC-ICP-MS analysis but the spatial resolution allows us to identify the new growth reliably in Raman spectroscopy, link the structural differences, and analyse well defined locations which is paramount to the ideas in the paper and would not be possible using bulk techniques.
Anonymous Referee: As a very basic approach, I would have expected to find a prediction of the \(\Delta^{11}B\) difference between natural samples and the high pCO2 treatment. Following the boron isotope fractionation factor of Klochko et al., the \(\Delta_{\text{bor}}\) values and environmental and experimental conditions, one can predict \(\Delta^{11}B\) of the coral skeleton in the high pCO2 treatments should be 1.8‰ 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 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.

Answer: We would like to apologize for reporting of pH and clarify the used pH scales. The mentioned pH in seawater scale is actually not a pH at seawater scale. We used an inappropriate abbreviation of seawater pH (pHsw) and we will change this in the manuscript. We introduced a new the abbreviation to clearly differentiate when we are talking about the pH of seawater or internal calcification pH and overlooked the resulting confusion.

The seawater pH for both the ambient and culturing conditions were reported as presented in Form & Riebesell 2012 as free scale. In the revised version we converted the pH to total scale and changed the abbreviation of seawater pH to pH_T (in total scale) though this does not affect our findings as differential to internal pH_c was calculated within the same framework. To convert \(\delta^{11}B\) to pH values, the conversion was exactly done as the referee suggested. We have added to the discussion on upregulation and provide all the necessary data.

Anonymous Referee: 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.

Answer: We agree with the referee that we still do not fully understand what governs and controls isotopic fractionation and incorporation in both inorganic and biogenic carbonates. While Sanyal et al. (2000) showed elevated \(\delta^{11}B\) inorganic calcite, the elevation is significantly lower (at pH_{NBS} 7.9 \(\delta^{11}B\) is approx. 19‰) than what we observed in the present study (for a pH_T range of 8.03 – 7.7 the \(\delta^{11}B\) ranged between 26.97-27.8‰) and other studies on cold-water corals in their natural environment (e.g. McCulloch et al. 2012 pH range: 8.1 – 7.77 and \(\delta^{11}B\) range: 24.5-28.69, Anagnostou et al. pH range: 7.58-8.05 \(\delta^{11}B\) range: 23.56-28.13). While we do not fully understand boron incorporation, importantly for this paper, corals have a strong biological impact on the boron isotope composition.

It is important to note that a recent study by Mavromatis et al. (2015) indicates that control mechanisms on boron incorporation in inorganic calcite are more complex than for aragonite and he follows “that calcite-based calibrations may be less reliable than aragonite calibrations for ocean paleo-pH reconstructions”. Overall, while we acknowledge that this is an important aspect, a detailed discussion of the Sanyal findings and the update with the current knowledge and implications for the interpretation of the data are outside the scope of our study.

Anonymous Referee: 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 harbour 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 \(\Delta^{11}B\) 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 CaCO3 (Sanyal
et al. 2000) records the same “vital effect” in δ11B 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 δ11B-deviation does not have anything to do with greater pH up-regulation at lower ambient pH. The inorganic precipitation experiments should be repeated but until we have contrasting evidence for inorganic CaCO₃, using δ11B 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 δ11B 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.

Answer: The referee is right, as δ11B is only an indirect measure in cold-water corals. Therefore we have clarified and added caution to our discussion. However, pH sensitive dyes also have significant own limitations and are not precise enough to elucidate minor changes in pH as discussed here. We also consider our interpretation corroborated by a comparison with tropical corals though of course these have different physiological and ecological impacts on the actual boron values. Holcomb et al. 2014 showed a pH up-regulation and relationship to δ11B; therefore we consider it is highly likely that this might be true also for cold-water corals.

Anonymous Referee: 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.

Answer: Given the large heterogeneity of boron within the coral, the standard deviation of the data is high, but due to the high number of analysis, the standard error is much lower. We have added significant amount of data to show that we can produce data which are reliable by comparing corallites from the same individual, by repeating a profile on the same specimen, and by comparing individuals from the same reef (see above). We are therefore confident about our data.

We acknowledge that our data and its precision would allow a difference of 0.3 pH units of up or downregulation at low pH as this is our error.

We did discuss the data of Blamart et al. 2007 and Rollion-Bard et al. 2011. Their values are significantly higher than data derived by other methodologies but they described the applied method thoroughly and used similar standards. Rollion-Bard et al. 2011 argued that a higher and variable (dependent on skeletal structure) fraction of boric acid is incorporated. Applying their model to our data using δ11B signature of the EMZ, the same individual would incorporate very different proportions of boric acid which is not likely given the broad range of literature on boron in corals in general. Therefore, we question this variable boric acid incorporation hypothesis (as underlined as well in the next comment of the referee).

As discussed above so far we lack a direct proof of internal pH up-regulation in cold-water corals. Thus
the extent of pH-upregulation is speculative but a likely trade in scleractinian corals and independent of seawater pH we observed the same $\delta^{11}$B an enhanced up-regulation ($\Delta pH_{cf}$ in the nature of 0.8-0.9 and for the CRSIII of 1.05).

**Anonymous Referee:** Furthermore, the authors should note that the entire NMR debate is 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.

Answer: We apologize for any misunderstanding in this matter in our manuscript and would like to clarify and revise our argumentation. As the reviewer stated, Rollion-Bard et al.'s 2011 study can measure only the coordination B in the crystal and it is not a direct measure of the incorporated form be it borate or boric acid. Nevertheless, in their case this mechanism was needed to explain the observed offset in $\delta^{11}$B and was used as indication that not only borate is incorporated (but of course is not a direct proof). As our data does not need this mechanism, we have significantly reduced the discussion to focus on the points we consider most important in this context and those we are in a position to address with our data.

The study by Sen et al.'s indicates a potential for boron to change its coordination: however, this argument needs to be treated with caution. In their study they induce phase transition from aragonite to calcite by heating the sample powder to 500°C. Such extreme treatment changes the B coordination (Sen et al. 1994) and therefore provides limited information on coordination changes during coral calcification under ambient seawater temperatures.

The Figure S5 in the supplements is not our main finding, and will be removed.

**Anonymous Referee:** Some minor issues that would benefit from greater detail: What is meant by “with precautions concerning its use for deep water corals” (page 6763)?

Answer: As described in McCulloch et al. 2012: he acknowledged that the isotopic fractionation factor of Klochko is derived for a temperature range of 25-40°C so not the temperature range where cold-water corals exist, but a change in temperature has a minor effect on the isotopic fractionation factor and thus, can be neglected.

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

Answer: We added a statement to the discussion explaining the consequences: *Our results raise a number of question: (1) can energy be reallocated to up-regulate the internal pH$_{cf}$ to a suitable level which would complicate the applicability of Lophelia skeletons $\delta^{11}$B record as a paleo-pH proxy given the small ranges of pH difference studies often aim to resolve.*

**Anonymous Referee:** 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 pCO2 experimental group?
Answer: Here we added an introductory paragraph that links the coral's physiology and also addressed Rodolfo-Metalpa's comment to include their recent published findings. In their recent paper they observed what we suggested here, that pCO₂ will not be critical when corals are not energy limited by provided with sufficient food.

Anonymous Referee: Page 6769: please discuss why one should be worried about changes in the organic layers when the CaCO₃ skeleton is as thick or thicker under undersaturated conditions compared to saturated conditions?

Answer: We will address this issue and discuss it in the text. The layered growth of biogenic organisms is a prominent feature and suggests a strong biological control of growth. Thus, a less clear banding could indicate that OM formation is compromised. Whether this is worrying or not, we cannot answer to date. Further studies are necessary to address the role and function of layered growth.

Anonymous Referee: Table S1 mixes commas and periods, please choose one for all.

Answer: We changed this in the revised version.

Anonymous Referee: 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.

Answer: We changed this in the revised version.

Anonymous Referee: 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 colour, e.g. blue.

Answer: This is a misunderstanding, the reference to Fig. S2 is the paragraph below and not part of the figure caption. We will change the font size to clearly differentiate between capture and main text. The operator symbols will be changed in the revised version as well as the colour of the asterisk in Fig. S4.

Referee Sebastian Hennige:

Referee Sebastian Hennige: Specific points: It is vital to include all the method background needed to interpret the results. Presently, it is hard to see how many samples were used in each condition and thus how much confidence we can have in results. This is crucial since the authors state how variable the samples are.

Answer: In the revised version we will provide more details on the method background. In respect to the stated variability we would like to provide some clarification. We have added significantly more boron data, showing that we can generate reproducible data in one corallite, in two branches of the same specimens and between specimens (see discussion above). This data also provides a caution that it is fundamentally important to compare specimen with similar growth history (as confirmed by Raman) to compare results in such studies.

Cold-water corals show δ¹¹B and other elemental heterogeneities within the early mineralizing skeleton (including EMZ like structure in the theca wall). To overcome this heterogeneity, studies using cold-water corals to trace seawater pH limit the sampling to the outer thecal wall and integrated larger skeletal areas. Cold-water corals do not grow fast and thus, to evaluate their δ¹¹B change with changing seawater high-spatial resolution techniques need to be applied to only sample material that was formed during the culturing period. Main growth occurs at the polyp tip, where the thecal wall is still very thin and predominantly formed by primary skeleton. This area is normally avoided in boron
studies and underlies very different incorporation mechanisms.

Referee Sebastian Hennige: Since the controls for this study are natural samples (i.e. not lab grown), it is not feasible to state that differences (or lack of) are due solely to ocean acidification. For a true control you would need samples grown under ambient conditions in the laboratory, as changes in biomineralisation could be a lab effect (i.e. possibly due to feeding regimes).

Answer: Very likely we sampled also skeleton that was grown during the culturing since the corals were kept 3 month under control conditions in the culturing facility in Kiel prior to the staining and experimental start. However, we agree that we do not have a proper control (also see comment above) and it would have been ideal to compare to corals grown under similar feeding regimes and also under food availability the corals experience in the field. Nevertheless, what we can show is that corals can enhance H+ pumping and reach the same internal pH as in the nature under elevated pCO₂. We do not know whether regular feeding allowed this corals to up-regulate higher.

Referee Sebastian Hennige: The results that organic matrix layers are less distinct in high CO₂ treatments are very interesting, but the discussion should be wider to consider that factors other than CO₂ could cause this but the discussion should be wider to consider that factors other than CO₂ could cause this.

Answer: The less distinct organic bands are indeed interesting, but it was not the focus of our study and we have just qualitative observations and not quantitative data. Future work shall address this topic in more detail, but the discussion on what potential other factors can drive changes in the organic matrix arrangement is beyond the scope of our study.

Referee Sebastian Hennige: Results: You state that the skeleton growth was variable (from how many samples and how were they compared?) and that there was no change in strength or structure. However, you did not measure strength, and the structure analysis is based solely on pictures. A table of measurements and some analysis would greatly strengthen this section.

Answer: We apologize for using the term strength, which we did not analyse. In the revised version we will just refer to structural changes and add more data.

Referee Sebastian Hennige: Discussion: You state “Tomographic analyses clearly showed that the morphology of Lophelia skeletons are highly variable and does not change under high CO₂ even in undersaturated waters, i.e. there is no morphological indication of a stress response.”, and “the template of size and shape of the corallite, does not change between treatments”. These conclusions are not really supported by the data. You do not have a proper control for comparison, and the growth under elevated CO₂ is not in newly grown corallites, but short extensions and thickenings of existing corallite(s). Since the extension just seems to be a corallite tip, it is unclear what you are basing this interpretation on. How many corallite tips were compared and how did you compare them?

Did you measure any sizes? Since all the growth you recorded were extensions of existing corallites, would it be influenced by the form it was already taking (i.e. continuing to grow through established EMZs?). Provision of quantitative measurements of key corallite characteristics in a table, along with more clearly defined methods would greatly strengthen this section and the paper as a whole.

Answer: Concerning prior corallite size see comment above. We added size measurements to the revised manuscript and a clearer indication of where samples were taken and how many comparisons were performed. We agree that we do not have a proper control as such but of course this is impossible in cultures as each individual has their own growth history and therefore comparing before and after staining as we have done can also be influenced by other factors. We will explain this clearly and will be more careful in the interpretation and discussing of our findings.
Comment Rodolfo-Metalpa:

I found this ms interesting. It adds new evidences on the ability of CWC to calcify and resist to OA; therefore it should be published after correction. I would like to add only a couple of comments to them raised by Reviewer #1. I think that although previously published by Form & Riebesell, details of the experiments should be fully reported in the ms. For instance, since F&R found a decrease in the coral growth in the short and acclimation in the long-term experiment, it is not clear which samples Wall et al used. This is an important aspect when the sensitivity of the coral to OA was discussed. In addition, since Wall et al used the same samples than F&R, some measurements made in this previous paper should be discussed by Wall et al in the light of their new findings. Method P6761 "and after a 3 month acclimatisation period they were stained using Alizarin Red S". Do the Authors mean after 3 months at experimental conditions?

Answer: The corals were kept in culture under control condition.

Rodolfo-Metalpa: Discussion. Food availability. With regard to the potential role of food in the resistance of CWC to OA, I invite the Authors to improve the discussion using more accurately Results from McCulloch et al 2012. Also our recent contribution by Rodolfo-Metalpa et al 2015 (Global Change Biology) could help the discussion about this matter.

Answer: Thanks for pointing to your recent publication. We changed the discussion in this matter and more accurately discussed McCulloch et al. 2012. In addition, improved the discussion on the food availability discussing this new findings.


Sen, S., Stebnins, J. F., Hemming, N. G. and Gosh, B.: Coordination environments of B impurities in calcite and...