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Biogeosciences Discussions

Interactive comment on "Effects of seawater pCO₂ changes on the calcifying fluid of scleractinian corals" *by* S. Hohn and A. Merico

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General reply

The reviews that we received were very constructive and will help us to strengthen our manuscript both scientifically and in terms of clarity of meaning. Thus we propose a number of revisions. Please find enclosed detailed responses to reviewer concerns and descriptions of the changes we propose and their scientific justification.

Anonymous Referee 1

General comments:

The authors present a model of coral calcification that aims to simulate how coral calcification responds to rising CO2. The model computes the kinetic reactions of carbonate chemistry and the flux of ions in and between four different model compartments: the seawater, the coral tissue, the coelenteron and the calcifying fluid. A model such as this is potentially very valuable as it attempts to provide a physiological, mechanistic explanation to why corals calcify more slowly under ocean acidification. As the authors point out, corals exert significant biological control over physio-chemical conditions at the site of calcification, so why changes in seawater chemistry (the growth medium) should effect the calcifying fluid remains an important question in ocean acidification research. The authors have constructed their model in such a way that it reproduces measurements of Ca2+ and pH in the calcifying fluid made by microelectrodes under seawater conditions of pH8.2 (Al-Horani et al. 2003. Mar Biol). The microelectrode data of Al-Horani is the only set of continuous light-dark data currently available that provides the kinetics necessary with which to test the model. Whether one agrees with the set up of model or not, it integrates a certain amount of what is known about the physiology underlying coral calcification and provides a useful platform with which to generate and investigate hypotheses about the mechanisms underpinning coral calcification. Although the overall ideas and approach underlying the model potentially make it a valuable contribution to the literature, there are some issues related to fundamental physiology that could be be addressed/ improved. The biggest concern relates to the values of tissue pH which are currently way above the range of physiological norms for any animal, including coral. I discuss this issue further in specific comments. Other issues relate to confusing terminology and underrepresentation of the literature, which are also discussed in my specific comments. I hope these issues can be addressed in a revised paper.

We thank the reviewer for the positive comments regarding the importance of our modelling study and for the very constructive suggestions on how to improve the manuscript. We take the points concerning the tissue pH, the confusing terminology, and the underrepresented literature, and we have dealt with these issues in the follow-

ing.

Specific comments:

Title: The title is a bit misleading. The authors carry out pCO2 perturbation experiments with the model, but they only show how this effects calcification. They do not give a figure that shows changes in the calcifying fluid under pCO2 perturbation. The title should be modified.

Given that our ultimate focus was on how pCO2 changes affect the overall calcification rates, we agree that the title might appear a bit misleading. We therefore propose a more general title in a revised version of the manuscript that should better fit our study. The new proposed title is: "Modelling coral polyp calcification and its relation to ocean acidification".

P. 2656, line 5: Not all projections of coral reef futures agree with this (see recent publication by McCulloch et al. 2012 Nature Climate Change. doi:10.1038/nclimate1473). It may be better to say that OA acting with other anthropogenic stressors is expected to cause disastrous effects on reef ecosystems.

Agreed. We propose to revise the concerning sentence as follows: "It is expected that ocean acidification in combination with other anthropogenic stressors will cause severe decline in coral abundance by the end of this century, with associated disastrous effects on reef ecosystems."

P. 2656, line 10: the term "calicoblastic layer" used for the fourth compartment is misleading. "Calicoblastic" refers to a cell type that makes up the aboral ectoderm, so "calicoblastic layer" could be mistaken for this epithelium. The authors are referring to the "subcalicoblastic layer or medium", the term used by Al-Horani et al. 2003 (on whose data the model is based), Venn et al. 2011. PLoS ONE 6(5): e20013. doi:10.1371/journal.pone.0020013, and others who have worked on this layer. Elsewhere the authors use calcifying fluid and they should stick this term or use subcali-

coblastic layer/ medium.

Re-reading our manuscript in light of the comments it is now clear that our original wording in terms of "calicoblastic layer" is misleading and the reviewer is quite right to pick us up on this. To avoid potential misunderstanding and for consistency, we will replace "calicoblastic layer" with the more appropriate "calcifying fluid" throughout the manuscript.

P. 2658. The authors explain how corals metabolically control the composition of the calcifying fluid. Because the model isn't purely geochemical and attempts to integrate coral physiology, a more detailed and more accurate introduction of the physiology underlying how corals modify the calcifying fluid is needed here. It would be helpful to the non coral specialist reader in evaluating the model. Of course, not every aspect of coral physiology can reasonably be included in the model, but the authors can explain what is and isn't included in the next section, "model description".

The reviewer is of course correct to reason that not every aspect of coral calcification discussed in the literature can be considered in the model also because in some cases data important to mathematically describe the concerned processes are not available. We will clarify this point in the revised manuscript and, following the more specific suggestion regarding coral physiology, we will extend the introduction to describe how the organic matrix can facilitate crystallisation and we will explain why we have not considered this process in the model. We will furthermore mention the paracellular pathway, besides the transcellular passage, and we will explain why the model only includes the transcellular pathway. Further details below.

As the model deals with coral tissue is would be relevant to point out here that the calcifying fluid is separated from the surrounding seawater by four cell layers, an oral ectoderm and endoderm and an aboral endoderm and ectoderm, (the latter cell layer being the calicoblastic cell layer).

Agreed, we will make this change.

It should be mentioned that evidence exists for both paracellular and transcellular passage of ions and molecules from the seawater (including Ca2+) to the calcifying fluid (e.g. Tambutte et al. 2011, Proc R Soc B, doi:10.1098/rspb.2011.0733). (The authors only consider the transcellular route and they can state this in the next section (model description).

Agreed, we will introduce this pathway. Further comments below.

The authors should mention (briefly) that corals produce an organic matrix from the calicoblastic cells at the tissueskeleton interface (reviewed recently Tambutte et al. 2011, J Exp Mar Biol Ecol. Volume 408, Issues 1–2, Pages 58–78).

We will describe this hypothesis in the introduction. However, the idea of an organic matrix comes from the observation that organic molecules are incorporated in the crystal structure, although it has never been shown that these molecules have a structuring function or that they enhance the crystallisation rate in the calcifying fluid. Holcomb et al. (2009), showed that the banding structure in the coral skeleton, which was previously attributed to the incorporation of organic molecules, thus supporting the idea of an organic matrix, is also present in inorganically precipitated aragonite. In our opinion, the hypothesis of an organic matrix remains speculative and the potential enhancement of crystallisation rates has not yet been quantified. So we conclude that it is safer to consider the "bio-inorganic" model described by Tambutte et al. (2011) in which the "incorporation of inorganic molecules into the skeleton is a by-product of rapid crystal growth" (see page 65 in Tambutte et al. 2011), at least until further knowledge will be available on the role of an organic matrix. We will clarify this in the revised manuscript.

Also, the authors should mention that the calicoblastic epithelium secretes enzymes e.g. carbonic anhydrase that facilitate the interconversion of the dissolved inorganic carbon species. Again it can be stated that for simplicity the addition of organic molecules to the calcifying fluid is not considered in the present model.

Agreed. We will mention the activity of carbonic anhydrase (CA) in the calcifying fluid

in the revised manuscript. In particular, we will refer to the study of Moya et al. 2008. As the reviewer suggests, we will mention that for the sake of simplicity the role of carbonic anhydrase is not included in the model. We will also point out that our model solutions compare very well to observations, suggesting that the model includes the most relevant processes.

The authors already discuss how pH is elevated in the subcalicoblastic medium (although they should perhaps reference pH imaging of the subcalicoblastic medium by Venn et al. 2011 PLoS ONE 6(5): e20013. doi:10.1371/journal.pone.0020013) and the potential role of the proton-calcium antiporter. Here it would be better to introduce that evidence for a Ca2+ ATPase comes from the biochemical study by Ip et al. 1991, Mar Biol. and molecular evidence by Zoccola et al. 2004, Biochim, Biophys Acta, rather than later in the "model description".

Agreed. We will add this reference in the manuscript. Since we will extend the Introduction to provide a more detailed picture of coral physiology, information on how pH is elevated in the subcalicoblastic medium and on the potential role of the proton-calcium antiporter (the Ca2+ ATPase) will fit perfectly into the revised Introduction.

Lastly, the authors do not mention the existence of a diffusive boundary layer (DBL) between the coral tissue and the surrounding seawater that influences the exchange of ions between the coral tissue, coelenteron and the growth medium. There are quite a few papers that describe the DBL (e.g. Shashar N, et al. 1993. Biol Bull 185: 455–461., Mass T, et al. Proc Natl Acad Sci U S A 107:2527–2531).

We agree that a diffusive boundary layer has an important influence on the exchange of ions between coral tissue, coelenteron and growth medium. However, the mentioned articles are related to oxygen fluxes and oxygen is not relevant to our study. We therefore believe that this aspect does not need to be mentioned in our manuscript.

Ok, as previously stated not everything can be included in the model, the task is daunting, but at least the reader should know what important aspects of coral physiology are

and are not included in the model.

We agree. A mathematical model is an approximate representation of a natural system and should be based on quantified processes. This model is the first of its kind and will hopefully sparkle a constructive dialogue between experimentalists and modellers to further improve our understanding of coral physiology. However, as already mentioned, we agree to expand our introductory section to include more information on coral physiology and we will explain why certain processes have not been considered in the model.

P. 2658, line 24. The four compartments proposed by the authors do not relate to the 4 compartments identified by Tambutte et al. 1996.

Tambutte et al. (1996) identified: 1) an efflux compartment for calcium ions considered to represent the coelenteron, 2) a NaOH soluble pool, which they proposed to represent the tissue, probably also including the calicoblastic epithelium, 3) a small labile skeleton pool representing the calcifying fluid, and 4) a bulk skeleton pool. The structure of our polyp model is inspired by the work of Tambutte et al. 1996 (cf. Figure 8, page 1037), although our seawater pool (or growth medium) is represented by a separate compartment. We combined the calicoblastic epithelium cells with the rest of the tissue so that the NaOH soluble pool includes all four different tissue layers. Seawater was not treated as a separate compartment in Tambutte et al. 1996 because their focus lied on the coral organism. We therefore propose to change the concerned passage as follows "...in four different model compartments (inspired by the work of Tambutte et al. 1996) and is...".

P. 2659- model description. As stated above the authors do not consider a paracellular pathway. This is a potential route by which seawater bicarbonate and carbonate reach the site of calcification. The authors already clearly state they are only considering CO2 diffusivity over the membranes and transcellular bicarbonate transport, but they should at least introduce the paracellular pathway earlier in the introduction and say it

is not included in this model.

The hypothesis of a paracellular pathway is based on the observation that a CaCO3 staining dye (calcein) makes its way from the outer growth medium to the extracellular calcifying fluid, staining the freshly precipitated aragonite (Tambutte et al 2012, Proc R Soc B, 279, page 19-27). Since this dye is known to be membrane impermeable (because of its hydrophilic properties), its appearance in the calcifying fluid is explained by a flux through the intercellular space. We agree to introduce the description of this pathway in the introductory part of our manuscript to present a complete description of the current understanding of coral calcification. There are, however, at least two reasons why we did not include the paracellular pathway in our model. First, as acknowledged by Tambutte et al. 2011 (page 65): "Whether the ECM '(extracellular calcifying medium)' is closed to the outside environment or open to it is still debated." Given the uncertainties, we decided to exclude this pathway and investigated if the model is still able to produce the observed changes in the calcifying fluid. Second, if the intercellular space is permeable to dissolved ions, then the flux between the calcifying fluid and the seawater (or the coelenteron) should be described either by diffusive transport or by advective transport. In both cases, the model would require the rate of the transports, but unfortunately these rates are presently unknown. As suggested by the reviewer, we will describe the paracellular pathway in the introduction and we will explain the reasons for excluding this process from our model.

P. 2659. If no paracellular pathway is being considered, why does figure 1 show a direct connection between the coelenteron and the calcifying fluid (termed calicoblastic layer in the figure) that does not pass via the tissue?

Good point. We will remove this arrow from the figure in the revised version of the manuscript.

P. 2663. Results, tissue. The model does not incorporate reasonable levels of intracellular pH. In the model, pH in the tissue varies between about 8.3 and 9.3 which are a

long way above physiological tissue pH of animals and plants. Unlike calcium, which the authors state cannot be constrained due to lack of concentration data, the range of intracellular pH in the light and dark has been characterized in coral cells from the endoderm layer (pH 7.1-7.4) and the calicoblastic layer (pH 7.4 in the light and dark) (Venn et al. 2009, PNAS. 106(39):16574-9. and Venn et al. 2011. PloS ONE. e20013. doi:10.1371/journal.pone.0020013). A realistic physiological model must incorporate values of pHi at least somewhere close to these values.

This is a very important aspect, which we admittedly overlooked in our original manuscript, and we thank the reviewer for pointing it out. We have now refined our model runs to obtain reasonable levels of intracellular pH. The refinement was achieved by changing the initial values of the state variables in the tissue. We especially reduced intracellular total alkalinity. Intracellular pH now lies between 7.1 and 7.4 and is therefore within the expected range. The adjustment of intracellular pH affected the carbonate chemistry in the tissue and the CO2 fluxes between the compartment boundaries. We therefore had to re-adjust and re-tune the model parameters to produce good fits to the observations of Al-Horani et al. (2003). With a more realistic tissue pH, the model results for the calcifying fluid have improved substantially (see Figures 1D and 2D). This is mainly due to the more realistic fluxes of CO2 between the tissue and the calcifying fluid that strongly influence the carbonate chemistry in the calcifying fluid. However, while the adjusted fluxes from the coelenteron into the tissue are now in accordance to the strongly controlled calcium concentration in the tissue (see Figure 1B), they are too small to induce appreciable changes between light and dark phases in the calcium concentration and the pH of the coelenteron (see Figure 1C and 2C). We will obviously revise the manuscript and the concerned figures accordingly.

P. 2664. Section 3.5. Calcification over time. The authors should remark that no calcification is observed in the dark and this doesn't really represent the biology very well as numerous studies show that corals continue to calcify in the dark. Estimations of the degree to which light "enhances" calcification vary, but are reviewed in Tambutte

et al. 2011, J Exp Mar Biol Ecol. Volume 408, Issues 1–2, Pages 58–78.

Since the experiments of Al-Horani were performed on a very short time scale and light was simply switched on and off, we considered reasonable in the model to simply switch off the ion transporters in the dark and to switch them back on in the light to mimic the observed response. As we already discussed in the original manuscript, this is of course a simplification given that in a real polyp the ion pumps will probably still be supported by energy supply via mitochondrial respiration. In the short time scales of the simulated experiments, however, our assumption appears well founded. Furthermore, in agreement with Tambutte et al. (2011), the modelled calcification rates in the dark decline to become one or two orders of magnitude smaller than in the light. To avoid potential misunderstandings, this aspect will be better emphasized in the revised manuscript. Also note that the diffusion of CO2 resupplies carbon to the calcifying fluid in the dark, allowing further (albeit slower) calcification. This point will be appropriately clarified in the revised manuscript.

P. 2666. Line 27. Although the authors state that little data exists for calcium concentrations in the tissue, they state that the pathways for calcium transport are relatively well known. This isn't really the case and the references the authors give only relate to molecular and invitro characterization of the Ca2+Atpase in the calcioblastic cells. The pathway of calcium from seawater through the tissue is not well understood. It would probably be better to remove this phrase.

With this statement we indeed refer to the characterization of the Ca-ATPase, the calcium transporter that mediates the passage of calcium ions over the cell membrane. This protein has been sequenced (Zoccola et al 2004), its presence in the calicoblastic epithelium has been proved (also Zoccola et al 2004) and its activity and kinetics have since long been measured (Ip et al 1991). This is much more than what is known for example for coccolithophores, a thought that lead us to conclude that at least the transport of calcium over the cell membrane is (in comparison to coccolithophores) relatively well understood in corals. It is not yet known, however, what happens to calcium in the

cytoplasm, how calcium is transferred from one cell layer to the other, and what role the mesogloea plays in the calcium transport (Marshall et al. 2007). We therefore propose to change the concerned sentence as follows: "The inorganic carbonate chemistry is not affected by major uncertainties (Zeebe and Wolf-Gladrow, 2001; Riebesell et al., 2009) and even the mechanism of active calcium transport into the calcifying fluid is relatively well understood (Ip et al., 1991; Allemand et al., 2004; Zoccola et al., 2004).

P. 2670. Line 17. It would help the non coral specialist reader if the authors provided one of the comprehensive reviews on coral bleaching e.g. Douglas 2003. Marine Pollution Bulletin 46, 385-392.

Thank you, we will add this reference.

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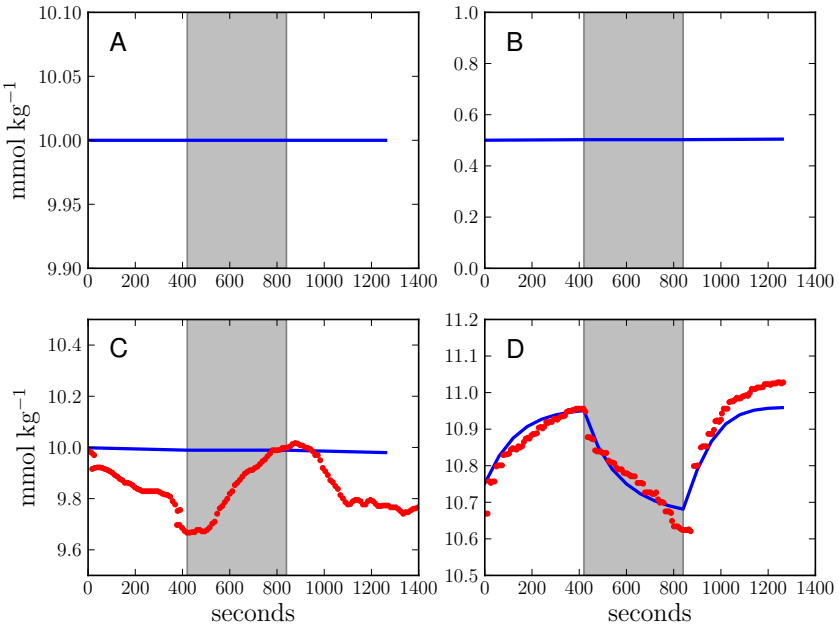

Fig. 1. Calcium ion concentrations in the four model compartments over time (A=seawater; B=tissue; C=coelenteron; D=calicoblastic layer).

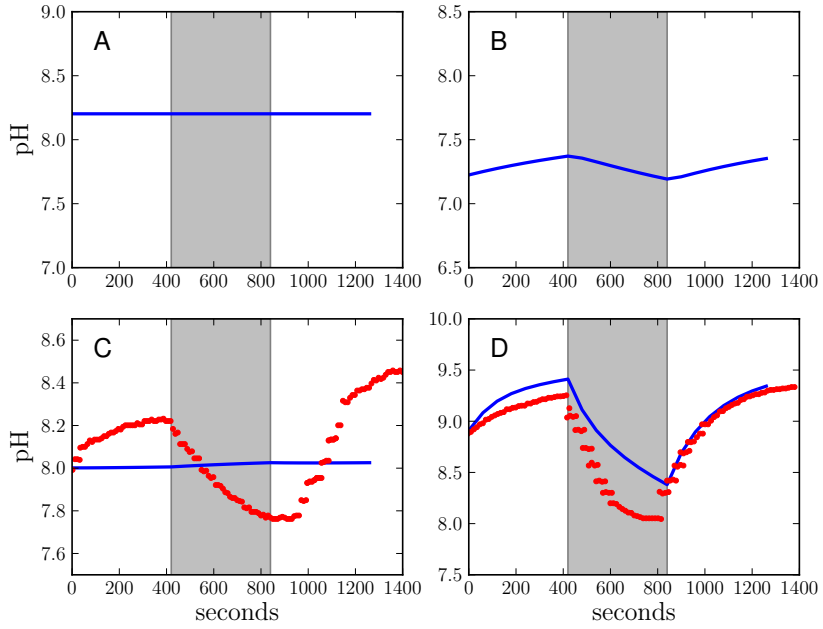

Fig. 2. pH in the four model compartments over time (A=seawater; B=tissue; C=coelenteron; D=calicoblastic layer).