Author Response to Anonymous Referee #2

Summary of Reviewer's Comment #1:

The lack of data supporting oxalate in the skeleton poses a problem to the validity of the model

Author's Response #1:

Rather than jumping straight to the (endpoint) aragonite coral skeleton in search of supportive evidence, I think it is beneficial to begin my defence of the proposed function of calcium-oxalate from the (oft ignored) standpoint that the process of coral biomineralisation is first and foremost a narrative based around the ability of a coral to maintain (and manipulate) a 'materials solution' between its calicoblastic epithelium and existing skeleton; which subsequently permits strong biological control over a phase transition from solvated state (Ca^{2+}/ CO_3^{2-} ions) into a crystal ($CaCO_3$) lattice. As reviewed by De Yoreo and Vekilov (2003), a very general and useful construct for thinking upon such phase transitions is the 'energy landscape'. The 'energy landscape' concept highlights that crystallization necessitates a phase transition through which matter is transformed from a state of high free energy in solvated state to one of low free energy in the crystal lattice. All aspects of a crystal, including its phase, habit, growth rate and orientation are controlled by the depths and shapes of the energy minima. By varying the heights of the barriers, the growth kinetics can be controlled, and non-equilibrium final or intermediate states can be selected. From this standpoint, it can be understood that corals modulate aragonite crystal growth by manipulating energy landscapes. And here (in his manuscript), I have specifically proposed that the controlled, biologically-mediated production of a calcium-oxalate precursor phase

(and its introduction into the 'materials solution') is important for lowering the energy minima required for $CaCO_3$ crystal nucleation.

By $CaCO_3$ crystal nucleation, I refer to the process of generating a $CaCO_3$ crystal lattice (new phase) from a solution of Ca^{2+}/ CO_3^{2-} ions (old phase) whose free energy has become higher than that of the emerging phase. Nucleation occurs via the formation of small embryos of the new phase inside the large volume of the old phase (De Yoreo and Vekilov, 2003). A prominent feature of nucleation is metastability of the old phase, i.e., the transformation requires passage over a free energy barrier (Kashchiev, 1999). One potential process to combat such metastability, is to increase the level of supersaturation (of $Ca^{2+}$and CO_3^{-2} ions) – promoting so-called *homogenous nucleation* (De Yoreo and Vekilov, 2003). Nucleation can however occur at lower levels of supersaturation if a seeding material is added to the 'materials solution' – so-called *heterogeneous nucleation* (De Yoreo and Vekilov, 2003). The presence of a foreign (heterogeneous) substance can exert strong control over nucleation because the interfacial energy between a crystal nucleus and a solid substrate is often lower than that of the crystal in contact with the solution (Mutaftschiev, 1993). This is because the molecules of the crystal can form bonds with those in the substrate that are stronger than the bonds of solvation. Because the enthalpic contribution to the free energy comes primarily from chemical bonding, stronger bonds lead to a smaller interfacial free energy. It is this chemical bonding – enthalpic energy process of a foreign surface that I propose is central to the involvement of calcium-oxalate in the heterogeneous nucleation, and subsequent growth and orientation of aragonite crystallites.

Based on the theory outlined above, I have proposed the following sequence of events (as is detailed in Fig. 6 of the manuscript):

1. Ca^{2+} ions (from the solvate solution) combine with secreted oxalate to nucleate (in a controlled/constrained fashion) calcium-oxalate crystals; as mediated by the presence of an organic matrix created from OPN- and HA-like material.
2. The bound Ca^{2+} ions then attract CO_3^{2-} ions (forming strong bonds that lower the required free energy for nucleation), and by having a sufficient concentration of these ions, induce nascent $CaCO_3$ nucleation.
3. Subsequent $CaCO_3$ crystal growth can then proceed in a manner typical of abiotic $CaCO_3$ precipitation from a supersaturated solution, with the initial crystal serving as a nucleation catalyst for formation of other crystals.
4. Notably, the expected fast dynamics and direct physical relationships in this multi-step process can be envisaged to form a template for epitaxial-type growth of the developing $CaCO_3$ crystals, wherein one crystal lattice overgrows (encrusts) another.

It is for this reason, I don't believe it is likely that you can expect to find (locate) calcium oxalate within the bulk aragonitic $CaCO_3$ coral skeleton. In essence, the calcium oxalate behaves as an instantaneous (triggering) catalyst for $CaCO_3$ deposition, but is overwhelmingly engulfed/overgrown – for all intensive purposes lost from detection; hence my description in the manuscript that it behaves as a 'ghost' product. The more profitable place to confirm its existence would be to consider the impact of factors that inhibit its production.

A valid question remains, 'why doesn't the pre-existing $CaCO_3$ skeleton provide the same catalytic (seeding) function?' I don't know the definitive answer to this, and suggest that it would be excellent question to formally test. I speculate that it has to do with the comparative crystal structure of calcium oxalate versus aragonite, and the resultant

strength of the bond that can form with the solvated CO_3^{2-} , especially at the lower saturation levels that exist during the 'dark calcification' phase (Al Horani et al., 2003). As outlined by the within-manuscript model description, at the high levels of supersaturation achieved during the 'light-enhanced' phase of calcification (Al Horani et al., 2003) any distinction would be minimised, and $CaCO_3$ could spontaneously deposit (homogeneous nucleation) upon all pre-existing skeletal elements in contact with the ECF.

Summary of Reviewer's Comment #2:

Very regular growth increments occur also in deep-water, azooxanthellate scleractinians. How precisely the "hypoxia model" may explain such regular succession of "seed"/"fibre" interactions in corals living in stable, deep-water (constant darkness) conditions without photosynthetic partners?

Author's Response #2:

Based on the slow extension rates of deep-water (azooxanthellae) corals the 'hypoxia model' predicts that levels of O_2-limitation stress in deep-water coral are much lower than for zooxanthellae corals in the surface zone. Yet, as correctly identified by Referee#2 the identification of regular succession of "seed"/"fibre" interactions in deep-water corals is predicted by the 'hypoxia model' to be driven by regular (cyclical) periods of O_2-limitation. Ultimately, it would be necessary to undertake the necessary oxygen measurements (e.g., with O_2 microsensors) to confirm the plausible nature of the 'hypoxia model' for deep-water corals, but it is not obvious to me that periodic O_2-limitation is not possible for deep-water corals.

Firstly, it is important to remember that the oxygen rich water in the surface zone does not mix readily with deeper water layers, and dissolved oxygen concentrations can be an order of magnitude lower even at depths of just over 100 m. Similarly, the stable environment of the deep ocean is unfavorable for diffusive mass transfer. Both these factors make it easy to argue that O_2-diffusion rates for deep-water corals will be considerably lower than for surface-water corals in the wave zones, i.e., the low ambient O_2 concentrations plus low rates of water movement in the stable, deep-water environment will considerably increase the thickness of the diffusive boundary layer surrounding deep-water coral, thereby increasing the potential for O_2-limitation during periods of high metabolic activity.

Secondly, it would appear reasonable to expect that the metabolic cycling of azooxanthellae corals will be regulated by entrained natural rhythms (e.g. circadian) that are known to exist in other cnidarians (see e.g., Vize, 2009) . These rhythms of maximum respiratory O_2-demand can be envisaged to drive periods of O_2-limitation stress given the low ambient supply of diffuse O_2.

Summary of Reviewer's Comment #3:

Why not to consider as hypothesis the simplest mechanism which would be charging and discharging mineral (ACC) bearing intracellular vesicles? This mechanism would also explain overall nanocomposite structure of biominerals (see also Mahamid et al. 2011: Cell Tissues Organs).

Author's Response #3:

I leave this suggestion for the referee to expand into a publication, I agree it that it does seem elegant – however, it is not immediately obvious to me that the temporary occurrence and stabilisation of an ACC phase would necessarily discredit the proposed model in any case? My personal observation with the history of coral biomineralisation research, is that the '*Why Questions*?' has been greatly overshadowed by the '*How Questions*?' simply because our 'tools' are better equipped to look at mineral end-products. Yet, I firmly believe that understanding the 'Why?' will instruct the 'How?' Hence my effort here to provide a conceptual model (= testable hypothesis) based around hypoxia being a fundamental driver within the process of coral biomineralisation. To the best of my ability I have endeavoured to explain how the existing data is commensurate with such a suggestion across multiple scales of observation (from genetics/subcellular – growth bands/structures - colony morphology – reef level deposition). Like all hypotheses, it remains valid only whilst it not disproven by any available evidence.

Summary of Reviewer's Comment #4:

The author includes speculations about significance of the model for understanding of ocean acidification (chapter 4.2.3) and as general calcification mechanism, for explanation of Cambrian "simultaneous" appearance of organized skeletons in many different taxa (chapter 6).... Wooldridge's model is an interesting starting point for discussion but the real discussion can only be continued using hard evidences and new experimental data from the areas outlined above.

Author's Response #4:

I can imagine that if these sections weren't included, there would be any number of referees' demanding that these known 'evidence constraints' be reconciled with the proposed biomineralisation model. Indeed, I concur with such sentiment. However, my intent with highlighting these issues in the current manuscript is simply to demonstrate that the model concept does, indeed, provide sufficient functionality to reconcile these important constraints – i.e., that the model concepts are not in opposition to these important 'evidence constraints'. I agree that in the event of a re-write, more attention should be given to highlighting the existence of other previously outlined (plausible) co-explanations/drivers exist, i.e., this manuscript does not need to discount their potential involvement.

Cited Literature

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