Author Response to Anonymous Referee #1

Reviewer's Comment #1:

It is not clear to me that the layered growth structure of a coral is a new discovery – Ogilvie 1896 clearly described a layered structure of growth lamellae surrounding dark points which seems to correspond to centers and banded fibers.

Author's Response #1:

The proposed biomineralisation model does not claim that a layered growth structure in scleractinian corals is a new discovery (and nowhere in the manuscript is this stated or inferred). Instead, the model endeavours to develop a new frame-of-reference (testable hypothesis) with which to reconcile the tradition mineralogical description (i.e., skeletal fibre growth = fan-like systems of moncrystalline fibres radiating out from "centres of calcification" foci), with new emerging observations that crystal fibre growth (and consolidation) includes a 'layered' morphology in which skeletal fibres with lengths of tens of micrometers consist of composite growth increments (bands) of aragonite approximately 2 to 5 μm long separated by thin organic-rich layers (see e.g., Cuif and Dauphin, 2005).

Reviewer's Comment #2:

The regions in which the highest rates of skeletal extension occur are the most exposed regions of the skeleton – septal tips, apical polyps, etc – all regions with relatively low densities of zooxanthellae and most exposed to water movement, thus the regions in which

hypoxia is least likely to occur. This disconnect poses a significant issue for the proposed model as I understand it and should be addressed, as it seems to me the regions with highest oxygen tension extend the fastest, thus hypoxia could be argued to repress extension.

Author's Response #2:

Nowhere in the manuscript do I prescribe the necessity for a direct spatial topology to exist between tissue regions with the highest levels of O_2-limitation (hypoxia) and skeletal areas of maximum extension, i.e., I don't discount the possibility that efficient transport mechanisms could exist to move organic (crystal seed precursor) material to the proximal (high calcifying) tips and edges.

One potential candidate mechanism is the movement of organic material within membrane-bound vesicles through mesogleal canals (ducts) associated with the gastrovacular system of scleractinian corals (Gladfelter, 1983; Gateńo et al., 1998; Rodriguez-Lanetty et al., 2005). Indeed, an elevated concentration of calcium in the mesogloea has been explained in terms of a transcellular transport mechanism for moving calcium to the site of calcification (Marshall et al., 2007). The structural logistics of a vesicle - mesogleal transport mechanism are feasible, since the large intercellular spaces in which the vesicles reside (prior to their known release into the calcifying space) are in direct contact with the mesogloea (Clode and Marshall, 2002). Furthermore, during periods of vesicle release, mesogleal extensions containing numerous vesicles are observed to ramify into the intercellular space between the calicoblastic cell-layer (Isa, 1986; Le Tissier, 1988, 1991; Clode and Marshall 2002).

I don't believe that it is necessary to speculate on this (or indeed other) potential transport mechanisms given the 'conceptual' nature of the present manuscript, other than to draw attention to their possibility, which I have endeavoured to do in the manuscript. In this regard, I draw the referee's attention to:

* The model description in Figure 6 [A(i)] wherein I write; "*During 'dark' calcification the CE is biologically active, with the cells becoming highly interdigitated, thereby creating large intercellular spaces into which the mesoglea extends and ramifies (Johnston 1980). Ca^{2+} ions, oxalate, and calcium binding substances (e.g., HA and OPN) are secreted into the ECF by the cells of the CE (either directly or via the mesogloea) – weighted most heavily towards the tip region.* ***The secretion mechanism(s) remains to be determined."***

****The manuscript's conclusion wherein I write; "*The conceptual nature of the model means that many of the proposed linkages remain to be described and tested in their entirety;* ***most notably relating to the dynamic functioning of the CE leading to the delivery of organic material at the skeletal-tissue interface***....."

Be that all as it may, I personally believe that it is entirely feasible that during non-photosynthetic (dark) periods, the net demand for respiratory O_2 (in excess of oceanic supply via diffusion) will be maximal at the rapid extending (tips and edges) regions of corals due to the excessive metabolic energy demands (from both the host and symbiont) in this biologically active region. In this regard, it is important to more carefully consider the symbiont population dynamics in this region. Whilst it is true to say that these proximal regions have low (instantaneous) zooxanthellae densities (Fang et al., 1989; Jones and Yellowlees, 1997), it is absolutely incorrect to suggest that this equates to low metabolic activity. Instead, the 'white' tips and edges of corals belies the fact that this tissue region

records the highest level of zooxanthellae turnover (= energy consumption) due to the combination rapid growth / algal division during the night-morning period (Fang et al., 1989; Jones and Yellowlees, 1997) and excessive expulsion rates during the midday-period (Jones and Yellowlees, 1997; Yamashita et al., 2011). See, Wooldridge (2012) for an explanation as to why high mass transfer regions (e.g., tips and edges) on corals are likely to equate with high symbiont turnover rates under modern oceanic conditions.

Reviewer's Comment #3:

The role of zooxanthellae in driving this process is further complicated by a lack of a difference in the respiration rates of some species of corals in the presence or absence of symbionts.

Authors Response #3:

I would need to see the coupled respiration and calcification data to comment more fully on this comment. I don't at all discount the fact that some non-zooxanthellate corals have high standing respiration rates (especially if mediated by increased temperatures), but would contend that they should also be matched by high calcification/extension rates (see e.g., Marshall and Clode, 2004).

Reviewer's Comment #4:

The lack of data supporting oxalate in the skeleton poses a problem, it should be clear as to whether the author considers extension to be primarily the product of oxalate crystals or

what their precise role is in the process – is this a stepped process – oxalate formation, then carbonate precipitation surrounding the oxalate (and if this is the case, why would it be the case when there is already an extensive aragonite skeleton which would presumably be a suitable site for further crystal growth with no need for another phase as the nucleation site, and why is carbonate formation shut down – particularly given that pH remains elevated which would presumably support continued carbonate deposition – indeed cyclic saturation states have been previously suggested to account for the layered growth of corals)? or simultaneously occurring processes with the precipitation of mixed carbonates/oxalates – and if this is the case, why is the oxalate formation considered critically important as opposed to a contaminating phase which cycles temporally leading to compositional variations? Is the oxalate thought to be lost during calcification as suggested on page 12639? or does it remain in the skeleton? Would azooxanthellate corals be expected to have consistently higher oxalate contents given they lack a daytime increase in internal pO2?

Author's Response #4:

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 raised by the referee, 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.

Referee's Comment #5:

It is unclear to me how this model provides new insight as to the fitness of different symbionts (pg 12641). Calcification is but one aspect of coral physiology, and its utility is always limited; in any measure of symbiont fitness, the translocation of materials between the host and symbiont must be considered as well as their ultimate fates, though the

difficulty of the latter often leads to parameters such as calcification being used as an imperfect proxy – this has always been the case regardless of the calcification model.

Author's Response #5:

I agree entirely with the sentiment of the referee, who is clearly well informed on the subject. Unfortunately, this is not universal within the coral reef research community, and indeed, a more widely voiced opinion that is that, 'Fast coral extension and calcification rates equate to an efficient, well functioning (=tight carbon cycling) coral-algae symbiosis', with the corollary that 'slow extension and calcification rates (e.g., as often seen with corals that harbour Clade D symbiodinium) must equate to an inefficient, poorly functioning (= weak carbon cycling) coral-algae symbiosis'. The proposed biomineralisation model highlights a potential weakness in this argument, but as the referee correctly suggests, this is just one of many weaknesses with the argument.

Referee Comment #6:

The evidence presented for this model in understanding for the effects of ocean acidification and the cambrian explosion is rather weak – I suggest eliminating these sections or substantially rewriting them.

Author's Response #6:

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.

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