

## ***Interactive comment on “Architecture, Growth Dynamics and Biomineralization of Pulsed Sr-Labelled *Katelsia rhytiphora* (Mollusca, Bivalvia)” by Laura M. Otter et al.***

**Anonymous Referee #2**

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Otter and colleagues examined the aragonitic shell of Veneridae *Katelsia rhytiphora*, which has compound composite prismatic ultrastructure in the outer layer and cross acicular ultrastructure in the inner layer, for studying shell architecture, growth dynamics and biomineralization processes. For this purpose, the authors employed comprehensive approach using various techniques including FE-SEM, EPMA, NanoSIMS, EBSD, Micro-Raman spectroscopy, and Thermal Gravimetric Analysis, on the specimens aqua-cultured in tanks treated by pulsed labeling experiments using Sr-enriched seawater. They also simulated Young's stiffness based on the EBSD crystallographic data. This MS provides excellent comprehensive data set and important insights related with biomineralization of Veneridae clam in which the shells are composed of

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representative two ultrastructures in bivalve mollusk. I felt that the MS is very descriptive, less deep insights, but I can imagine that it is an adequate way to present such a big dataset. Such comprehensive demonstration of the details of the shell will be very valuable for wide range of communities such as biomineralization, paleoceanography, and paleontology, so topics treated in this MS will largely appeal to a broad readership of Biogeosciences. However, I felt that there are some parts that should be improved before acceptance for publication, thus my decision is “Moderate Revision”.

Description and interpretation of the data which related to crystallography and biomineralization seems to be OK, however, discussion about elemental transportation was based on very weak evidence thus problematic. Especially, the evidences the authors based on is (1) fluctuation of gray contrast observed at the growth portion during the Sr-enriched labelling experiments obtained by BSE image, even though BSE contrast is unreliable method for quantifying Sr concentration, and (2) similar enrichment factor (Shell/Seawater ratios) in labelled and non labelled conditions in both ultrastructural layers acquired by EPMA analysis, while the way for presentation of this enrichment factor is not adequate for discussing the element transport. Because most of the discussion regarding biomineralization is good quality, and because the length of the MS is already enough, so I recommend to simply delete the contents related to element transportation.

I would like to also suggest to add a new schematic drawing for summarizing the biomineralization and shell formation mechanisms obtained by this study. SEM and EBSD pictures are of course very nice, but they are sometimes too complicated for readers. A simplified drawing will be very helpful for readers to grasp the main conclusion of this MS.

Major comments: Title: The authors not only examined the pulsed Sr-labelled portion of the shell, but also examined the shell comprehensively, so I recommend changing the title.

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P1, L24, L26-27, As mentioned above and below, the discussion of the element transportation is based on too weak evidence, so I recommend to deleting this part.

P3, L31, More detailed information of labeled seawater circulation is necessary. Did the authors use a single batch of seawater, or prepare labeled seawater every time for changing the water? How robust was the stability of the Sr concentration? The seawater renewing was performed constantly or done at once? Because the authors did not provide seawater composition, the Sr fluctuation, if exist, is suspicious. Changes in Sr/Ca ratio in seawater can easily produce Sr/Ca fluctuation in the shell. This is very important and critical for the discussion for the elemental transport mechanism.

P12, L1-17, I would suggest adding simulation data of Young's stiffness for two test cases, (1) Single aragonitic crystal, and (2) The same crystal arrangement, but have a random orientation of the crystals. Is it possible? The comparison between (1) and (2) will provide the contribution of complex 3D construction of multi-order unit of crystal arrangement, and that of between (2) and the results presented in the MS will provide a contribution of control of crystal orientation by bivalve, is this right? I am not familiar with the stiffness simulation, so I am not completely sure that this suggestion is pointing or not.

P13, L13, the "bright grey areas" must not be caused "by variation in Sr concentration". It is OK to say that the contrast between labeled and non-labeled part is caused by the Sr concentration changes, because this is validated by Sr/Ca analysis by NanoSIMS and EPMA. However, the variation within the labeled portion was not be assured. Can you see this fluctuation also in Sr/Ca map? The contrast of BSE image is not only induced by Sr concentration but also by density (mass number) and topography. As the authors discussed, organic concentration can even change the contrast of BSE. If the authors want to discuss Sr concentration variation, they should be based on Sr analysis, not on BSE image. According to this, the evidence for the discussion at P13, L19-23 relies on very weak evidence. Additionally, the authors did not provide Sr and Ca composition of seawater, so it is difficult to exclude the possibility that this variation

C3

is attributed to the changes in seawater composition.

P14, L8-29, "4.4 Revisiting the Concept of Ion Transport Pathways". I recommend omitting this section because this section seems to be based on very weak evidence as mentioned above comments. In addition to the unreliability of BSE as Sr indicator, similar "enrichment factors for Ca and Sr (Ca-shell/Ca-seawater and Sr-shell/Sr-seawater)" is not an appropriate parameter for discussing the elemental fractionation. This should be discussed by distribution coefficient  $(\text{Sr/Ca-shell})/(\text{Sr/Ca-seawater})$ . Judging from the data in Table3, the data does not seem to satisfy enough robustness for discussing this topic. The authors also ignore fractionation between EPF (if exist) and carbonate. This can also produce low Sr/Ca ratio in the shell, without changing the EPF composition. No evidence was also presented for justifying the ACC formation obtained in this study. So, overall this section is not supported by the original data, thus should be omitted.

P16, L1-6, Conclusion. The second conclusion is OK, but the first and third conclusions were not supported by the data presented in this MS, because of the reasons as mentioned above.

Minor comments: P2, L5-10, Organic macromolecules itself can also control trace element incorporation. See, Stephenson A. E., DeYoreo J. J., Wu L., Wu K. J., Hoyer J. and Dove P. M. (2008) Peptides enhance magnesium signature in calcite: insights into origins of vital effects. *Science* 322, 724– 727 Wang D. B., Wallace A. F., De Yoreo J. J. and Dove P. M. (2009) Carboxylated molecules regulate magnesium content of amorphous calcium carbonates during calcification. *Proc. Natl. Acad. Sci. U.S.A.* 106, 21511–21516.

P4, L14, Magnification is not necessary, because it will be ultimately depends on print or screen size.

P4, L26, What is "Phenom XL"? P5, L27, "DREMEL tool" is not adequate. Maybe you should provide information of producer company, or use general name?

C4

P13, L14, insert space between 6 and increment.

P15, L29, Why don't you add "EBSD"?

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