

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

Laura M. Otter et al.

laura.otter@mq.edu.au

Received and published: 13 March 2019

Answer to anonymous referee #2

We thank the referee for their constructive comments, which will provide a helpful basis for the revision of our ms in due course.

We appreciate that the referee feels that the way of presenting our large dataset is adequate. The referee noted that the ms is more on the descriptive side, and this is indeed a main in our work as this shell architectural type has never been studied at the micro to nano scale before. We see the work presented in this ms as a baseline for

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further work carried out on this type of shell architecture in the future, which necessarily needs to build on a detailed description.

In the following we are listing the referee's comments followed by our answer.

Referee comment: "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 aquired 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."

Answer: We agree that the discussion about elemental transportation is a more speculative part of the manuscript and after critically revisiting it, we have decided to shorten this part considerably upon revision. We also agree that BSE imaging per se is an unreliable method of quantification. This is exactly the reason why we went to great length to calibrate the grey scale of the BSE imaging by combining it with quantitative electron microprobe measurements using Wavelength Dispersive Spectrometry (WDS) with the same instrument and in the same session as carrying out the BSE imaging. Such calibration enables direct comparison of the grey scales in the BSE images with the quantitative data using WDS in the Electron Microprobe. Further down in the text, the referee agrees with us on this point.

Referee comment: "I would like to also suggest to add a new schematic drawing for summarizing the biomineralization and shell formation mechanisms obtained by this

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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.”

Answer: We will provide a schematic upon revision.

Referee comment: “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.”

Answer: We believe the current title already reflects the ‘comprehensive examination of the shell’ as stated by the referee. The purpose of the label is to provide markers in time for the study of the entire shell. We will however reorganise and add the term ‘shell’ to the title to specify the aim of the study. We suggest the new title to be: ‘Architecture, Growth Dynamics, Biomineralization and Pulsed Sr-Labeling of *Katelysia rhytiphora* shells (Mollusca, Bivalvia)’

Referee comment: “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.”

Answer: As outlined above we intend to shorten and focus this section in the ms and will rewrite the abstract to reflect this.

Referee comment: “P3, L31, More detailed information of labelled seawater circulation is necessary. Did the authors use a single batch of seawater, or prepare labelled 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.”

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Answer: For labelling, seawater was enriched in Sr by adding 4.380 g Sr-hexachloride per 10 l of seawater and was freshly prepared each time the water had to be renewed. Renewing the water was done at the start and in the middle of each labelling period. As we used natural seawater from two large 2 seawater storage tanks of 10,000 ltr capacity each and a high precision balance, precise to the third digit, we consider the Sr concentration data robust.

Referee comment: “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.”

Answer: Presenting the Young’s modulus for a single crystal will require the use of a different reference frame, namely the crystal structure, which is distinct from that used here, which is using the shell orientation. We believe that this could lead to more confusion for the reader rather than contribute to a better understanding. We will include a reference to an earlier publication from our group that shows the Young’s modulus for an aragonite single crystal as reference (Agbaje et al 2017). Depicting a randomly oriented fabric in a pole figure means that the aragonite crystallographic axes will be randomly oriented. Therefore, the elastic properties of the crystal would be averaged and the fabric would be isotropic. A pole figure depicting an isotropic orientation would show an even distribution across the entire pole figure and would therefore be very uninformative to the reader. We propose to add a sentence describing that a sample with random crystal orientation would lead to isotropic results.

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O.B.A. Agbaje, R. Wirth, L.F.G. Morales, K. Shirai, T. Watanabe, M. Kosnik, D.E. Jacob (2017). Architecture of crossed-lamellar bivalve shells: The Southern Giant Clam (*Tirana dears*, Roding, 1798). R. Soc. Open Sci. 4: 170622. <http://dx.doi.org/10.1098/rsos.170622>

Referee comment: “P13, L13, the “bright grey areas” must not be caused “by variation in Sr concentration”. It is OK to say that the contrast between labelled and non-labelled 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 labelled 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 is attributed to the changes in seawater composition.”

Answer: The resolution of the Sr/Ca maps obtained by NanoSIMS unfortunately do not allow to observe any variation at this spatial scale. As argued above, the Sr and Ca composition of the water, particularly during the labelling periods, is constant within analytical uncertainty and can thus be excluded as a source of grey scale variability in BSE. Neither did we observe growth irregularities (e.g. organic components). Topography and edge effects would not result in such regular patterns of grey scales as observed here.

The fine grey banding, however, also shows up in the Raman maps (e.g. Figs.3, S2) and as Raman is not sensitive to electron density effects this would exclude these as a cause for the banding. Furthermore, deconvolution of the Raman signal is consisted with variation in SrCO₃ concentration as underlying cause for the grayscale banding observed in BSE, as increased Sr concentration in aragonite results in peak broadening

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(Fig. 3) and peak shift of the main carbonate band to lower wavenumbers (Fig. S2) (Alia et al., 1997, O'Donnell et al., 2008, Ruschel et al., 2012). This is direct evidence for the correlation of lighter grey scales in BSE with higher Sr concentrations in the aragonitic shell. Upon revision of the manuscript we will clarify this connection between BSE and Raman analysis more.

References for this answer:

Alia, J. M., Mera, Y. D. de, Edwards, H. G.M., Martín, P. G., and Andres, S. L.: FT-Raman and infrared spectroscopic study of aragonite-strontianite ($\text{Ca}_x\text{Sr}_{1-x}\text{CO}_3$) solid solution, *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, 53, 2347–2362, 1997

O'Donnell, M. D., Fredholm, Y., Rouffignac, A. de, and Hill, R. G.: Structural analysis of a series of strontium-substituted apatites, *Acta Biomaterialia*, 4, 1455–1464, 2008.

Ruschel, K., Nasdala, L., Kronz, A., Hanchar, J. M., Többens, D. M., Škoda, R., Finger, F., and Möller, A.: A Raman spectroscopic study on the structural disorder of monazite–(Ce), *Mineralogy and Petrology*, 105, 41–55, 2012.

Referee comment: “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 $(\text{Sr}/\text{Ca-shell})/(\text{Sr}/\text{Ca-seawater})$. 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.”

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Answer: After reflecting on both reviewers' comments that this section is very speculative, we feel it is best to shorten this section considerably and to focus only on discussing the most likely ion transport pathway. In addition, we will use the distribution coefficients ($Sr/Ca(\text{shell})/Sr/Ca(\text{seawater})$) to outline our thoughts and change Table 3 accordingly. Including possible fractionation by any potential EPF is extremely speculative and is therefore not warranted. However, we will make sure that the revised version of the ms reflects any further potential fractionation by a (potential) EPF and tone down the assertive tone of this section.

Referee comment: "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."

Answer: We have listed four conclusions and believe the referee is referring to conclusion two and four as being too speculative. The revised ms will take this into account and this section will be re-written.

Referee comment: "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.

Answer: Thank you for pointing out the omission of these important works. This part of the discussion will be rewritten, also taking the comments referee #1 on board and we refer the readers to our answer to referee #1 for this issue.

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

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Answer: Thank you, mention of the magnification factor will be omitted in the revised version.

Referee comment: “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?”

Answer: Phenom XL is the product name of the SEM used in this study. Similarly, a DREMEL tool is the official name of this tool. The term SEM is mentioned in the same sentence with Phenom XL. We suggest adding the term ‘power tool’ after ‘DREMEL’ to make this clearer.

Interactive comment on Biogeosciences Discuss., <https://doi.org/10.5194/bg-2018-469>, 2018.

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