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Interactive comment on "Marine and freshwater micropearls: Biomineralization producing strontium-rich amorphous calcium carbonate inclusions is widespread in the genus Tetraselmis (Chlorophyta)" by Agathe Martignier et al.

Reply to reviewer n°2

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Dear Editor, dear Authors,
Thank you for providing me with the opportunity to review this interesting ms on the formation of amorphous calcium carbonate in the cells of micro-algae. Obviously, I am not a lacustrine microbiologist hence my comments are those of a person interested in carbonate biomineralization, metastable carbonate phases, and the role of elemental cycles in aquatic environements. Moreover, I have read the review of E. Couradeau and my below remarks do not iterate the – in my view – well justified criticism already brought forward. This allows me to place my comments at a higher conceptual level. Generally, my impression of this ms is very positive. This is modern, state-of-theart research dealing with a hitherto less than sufficiently well studied topic. From the perspective of a carbonate sedimentologist and a person interested in the interface between abiogenic and biogenic processes, however, I have a few general comments, some of which are, as indicated above, also editorial in nature and aim for a paper that is accessible to a wider readership.

1) I do not think that the abstract serves well to attract the interest of a wider readership outside of the specialized community performing focussed research in this field. Please consider to start the abstract with a topical statement on element cycles and the role of algae in this "game". The immediate focus on Tetraselmis leaves the reader with the impression of a somewhat narrow approach. I think the paper as such is much broader actually and the abstract undersells the significance of this story.

Authors' response:
We thank the reviewer for this suggestion. We will replace the first paragraph of the Abstract by the following text:
*"*Unicellular algae can play important roles in the biogeochemical cycles of numerous elements, particularly through the biomineralization capacity of certain species (e.g. coccolithophores greatly contributing to "organic carbon pump" of the oceans) and unidentified actors of these cycles are still being discovered. This is the case of the unicellular alga *Tetraselmis cordiformis* (Chlorophyta) that was recently discovered to form intracellular mineral inclusions, called micropearls, which had been previously overlooked. These intracellular inclusions of hydrated amorphous calcium carbonates (ACC) were first described in Lake Geneva (Switzerland) and are the result of a novel biomineralization process. The genus *Tetraselmis* includes more than 30 species that have been widely studied since the description of the type species in 1878.
The present study shows that many other *Tetraselmis* species share this biomineralization capacity…"

2) The Introduction, albeit often well written, is in part a bit unstructured or so it seems and I would like to see that the authors provide text regarding the aims of this paper. I guess that would be pretty standard and I know that many readers like to have an idea of the general direction the paper takes.

Authors' response:
We agree with this remark from the reviewer. We will try to give a better structure to the introduction. The last paragraph (Page 3, Line 11) already describes the research which is detailed in the manuscript. We will gladly complete this paragraph.

3) The results chapter is generally well-designed but in places transgresses the boundary between genuine data presentation and interpretation, perhaps the most commonly found criticism in reviews these days. That could be rather easily solved by using a header such as Data "Presentation and Interpretation" and by restructuring the text in a manner that physically separates (paragraphs) more descriptive text from more interpretative text. Again, by this I aim for a better accessibility of the text for the nonspecialized reader. Clearly, chapter 3.5 is more of an interpretation than a genuine data reporting. Please consider.

Authors' response:
We suggest to rename this part "Results and Interpretation". We agree to modify the structure of the text to try to separate more clearly interpretation from the results themselves, as suggested.

4) Discussion: I was – in places – a bit confused about the manner in which the authors jump between lacustrine and marine micropearls. Could you do the reader the favour and commence the discussion with a paragraph explaining the reader how you structure the text? After all, the title of this paper refers to lacustrine and marine case examples but I do not see any chapter in the discussion specifically referring to lacustrine micropearls (but there is one dealing with marine examples actually referring to freshwater ones in the first paragraph)? I am aware of the fact that you have published on lacustrine examples before and that you place the lacustrine and marine findings into context but this all seems a bit unbalanced and would clearly benefit from some form of a better structure.

Authors' response:
We definitely agree to write a first short paragraph in the discussion, explaining the structure of the text. Appart from that, we admit that our main idea when writing the discussion was to discuss the Tetraselmis genus as a whole, and we haven't been attentive to separate the marine from freshwater species. We suggest renaming part 4.1 "Marine and freshwater micropearls" as a first step. We could then distinguish clearly the marine micropearls from the freshwater ones by doing two separate paragraphs in part 4.1. For the other parts of the discussion (4.2 to 4.4), we do not think it is relevant to make that distinction, as they discuss the Tetraselmis genus in general, without reference to the species' habitats.

5) Generally, I found the literature cited on ACC somewhat "classical". These are clearly landmark papers worth citing but a series of more recent studies dealing with thermodynamic and kinetic and biomineralization aspects on how and why organisms secrete or induce amorphous phases seems absent or so I think. I am happy to provide references should the authors wish so.

Authors' response:

The various questions of the reviewers on this topic lead us to already plan to add the following references (see below) to the future revised manuscript. However, we thank the reviewer for his suggestion and we will also include the additionnal references he suggests to the revised ms.

Albéric, M., Bertinetti, L., Zou, Z., Fratzl, P., Habraken, W., and Politi, Y. The crystallization of amorphous calcium carbonate is kinetically governed by ion impurities and water. Adv. Sci., 5, 1701000, 2018.

Aizenberg, J., Lambert, G., Weiner, S., and Addadi, L. Factors involved in the formation of amorphous and crystalline calcium carbonate: a study of an ascidian skeleton. J. Am. Chem. Soc., 124, 32-39, 2002.

Dupraz, C. , Reid, R.P., Braissant, O., Decho A.W., Norman, R.S., and Visscher, P.T. Processes of carbonate precipitation in modern microbial mats, Earth-Sci. Rev., 96, 141-162, doi:10.1016/j.earscirev.2008.10.005, 2009.

Levi-Kalisman, Y., Raz, S., Weiner, S., Addadi, L., and Sagi, I. Structural differences between biogenic amorphous calcium carbonate phases using X-ray absorption spectroscopy. Adv. Funct. Mater.,12, 43-48, 2002.

Littlewood, J.L., Shaw, S., Peacock, C.L. Mechanism of enhanced strontium uptake into calcite via an amorphous calcium carbonate (ACC) crystallisation pathway. Cryst. Growth Des., 17, 1214-1223, 2017.

Mass, T., Giuffre, A.J., Sun, C.-Y., Stifler, C.A., Frazier, M.J., Neder, M., Tamura, N., Stan, C.V., Marcus, M.A., and Gilbert, P.U.P.A. Amorphous calcium carbonate particles form coral skeletons. PNAS, 114, E7670-E7678, 2017.

Politi, Y., Batchelor, D.R., Zaslansky, P., Chmelka, B.F., Weaver, J.C., Sagi, I., Weiner, S., Addadi, L. Role of magnesium ion in the stabilization of biogenic amorphous calcium carbonate: a structure-function investigation. Chem. Mater., 22, 161-166, 2010.

Rodriguez-Blanco J.D., Sand K.K. and Benning L.G. ACC and vaterite as intermediates in the solution-based crystallization of CaCO3. Chapter 5 in "New Perspectives on Mineral Nucleation and Growth", edited by Van Driessche A.E.S, Kellermeier M., Benning L.G. and Gebauer D. Springer, 2017.

6) The chapter, brief as it might be, that I really miss is one providing the reader with information about the wider significance of the amorphous calcium carbonate with respect to carbonate cycles and elemental cycling in these water bodies. Could you provide back on the envelope estimates about the volumes of material that are cycled here and the temporal constraints (seasonal patterns)?

Authors' response:

Unfortunately, it is definitely too early to be able to estimate, even by back of the envelope calculations, the importance that this biomineralisation pathway might have in quantitative terms when the whole carbonate cycling in surface waters is considered. The timescales of

formation and the fate of the micropearls (dissolution and/or conversion to another mineral state) are yet unknown.

*Nevertheless, we will add the following paragraph as an answer to the first part of the question: "*ACC is an important actor in the biogenic carbonate cycle because it is a frequent precursor to calcite, as many organisms use ACC to build bio-minerals with superior properties (Albéric et al., 2018; Rodriguez-Blanco et al. 2017). For example, the precipitation of calcium carbonate in microbial mats, the Earth's earliest ecosystem, starts with an amorphous calcite gel (Dupraz et al., 2008), and the formation of ACC inside tissue could make coral skeletons less susceptible to ocean acidification (Mass et al., 2017)."

7) Conclusions: Please don't come up with a paper written in 2010 (Raven and Knoll) and refer to something that was considered non-existent at that time. I agree, eight or so years seem not long ago but in our hyperactive research environment, this is actually a long time and significantly more accurate and recent findings have been published since then.

Authors' response:
We agree that 2010 is not a very "recent" reference. Nevertheless, we have not found a more recent reference which lists all the known organisms having ACC intracellular inclusions. Additionally, we did not find any article mentioning a unicellular eukaryote organism known to produce intracellular ACC inclusions, apart from Tetraselmis. We would be very keen to read new articles on this subjects that we are not aware of.

Summing up: My comments aim to improve an already very nice paper and I clearly encourage the journal Biogeosciences to consider publication of this work. My comments are on a conceptually high level given that a technical review is already available and given that I would like to see a wider readership making use of the science documented here.
I hope my comments are of use to the authors.
Sincerely yours, Adrian Immenhauser