UNIVERSIDAD COMPLUTENSE DE MADRID

Facultad de Ciencias Geológicas
DEPARTAMENTO DE MINERALOGÍA Y PETROLOGÍA

Ciudad Universitaria
28040, Madrid (Spain)
Departamento de Mineralogía y Petrología

MSc. Pablo Forjanes
e-mail: *pforjane@ucm.es*

Prof. Dr. Lurdes Fernández-Díaz
e-mail: *lfdiaz@geo.ucm.es*

We express our acknowledgements to the reviewers for the insightful comments, which helped to improve the manuscript.

Below is a detailed reply to all the referees' comments and a description of all the *changes incorporated to the revised manuscript*. A copy showing these changes is attached with all the changes marked in red. We hope that the manuscript in its revised version should be acceptable for publication in ***Biogeosciences***.

Remarks by Referee 1:

> ***Remark 1***. *The paper provides very important, massive analytical documentation of more or less subtle alteration of biogenic aragonites in experimental diagenetic conditions. It provides direct comparison between short-term, high-temperature diagenetic experiments (Casella et al. 2017) and long-term, low-temperature experiments described in reviewed paper. The methods to assess some of these tiny structural modifications of the skeleton are very appropriate, especially the EBSD measurements and grain size statistical evaluation (combined with other characterization methods). However, diagenetic experiments at 90ºC (Bernard et al. 2017 Nat. Commun. 8: 1–10; Cisneros-Lazaro et al. 2022 Nat. Communications 13:113) suggest that even such theoretically stable biominerals like low- Mg calcite are subject of rapid diagenetic geochemical alteration which is not expressed in any structural changes. From this view point the structural stability is not a proof of lack of diagenetic imprint. This perspective should be outlined in the revised version of the paper (it will additionally append the authors conclusion that "during the diagenesis, most fossil carbonate hard tissues are probably overprinted, even if they do not show clear signs of carbonate phase change" (line 705).*

We acknowledge the reviewer's comment. We fully agree that structural stability is not a proof of lack of diagenetic imprint. However, in the work under consideration, we only study the diagenetic alteration of aragonitic biominerals. Aragonite is not the stable $CaCO_3$ phase under any of the experimental conditions considered in this study.

Therefore, its recrystallization into calcite should be the expected outcome after diagenetic alteration. Surprisingly, for all the biominerals investigated, bioaragonite recrystallization into abiogenic aragonite always precedes calcite formation during diagenetic alteration. This fact is discussed at length in the manuscript. We are currently preparing a second manuscript on the diagenetic alteration of mollusk shells made of aragonite, calcite, and aragonite/calcite. We can anticipate that calcitic hard tissues also undergo significant diagenetic overprint and extensive transformation into abiogenic calcite. In any case, to stress the concept that features of structurally stable biominerals can also become overprinted during diagenesis we have amended the manuscript by adding the following paragraph and the references below to the introduction section and the list of references, respectively:

"*It is noteworthy that biogenic calcite is also metastable with respect to abiogenic calcite, and calcite biominerals may undergo dissolution-recrystalization reactions that result in the diagenetic overprint of their isotopic notations, as recently experimentally demonstrated (Bernard et al., 2017; Cisneros-Lázaro et al. 2021, 2022).*"

- Bernard, S., Daval, D., Ackerer, P., Pont, S. and Meibom, A., 2017. Burial-induced oxygen-isotope re-equilibration of fossil foraminifera explains ocean paleotemperature paradoxes. *Nature communications*, *8*(1), pp.1-10.

- Cisneros-Lazaro, D., Adams, A., Guo, J., Bernard, S., Daval, D., Baronnet, A., Grauby, O., Vennemann, T., Stolarski, J., Baumgartner, L. and Meibom, A., 2021. Species-specific foraminiferal ultrastructures modulate surfaces available for diagenesis. *Microscopy and Microanalysis*, *27*(S1), pp.274-275.

- Cisneros-Lazaro, D., Adams, A., Guo, J., Bernard, S., Baumgartner, L.P., Daval, D., Baronnet, A., Grauby, O., Vennemann, T., Stolarski, J. and Escrig, S., 2022. Fast and pervasive diagenetic isotope exchange in foraminifera tests is species-dependent. Nature communications, 13(1), pp.1-11.

Remark 2. *Figures 6 and A13 shows comparison between pristine vs. 6 month altered skeleton of Porites sp.. The authors conclude in the caption (Fig. 6) that "No major changes can be observed between the pristine and the altered skeletal elements". However, in contrast to elongated aragonite biocrystals in "6a" that form the fibrous skeletal region (not the microcrystalline centers of calcification), it is clear that crystals in '6b" form domains that are larger than diameters of pristine aragonite biocrystals. These domains are also visible in EBSD band contrast maps (Fig. A13). As mentioned by the authors [line 580] "Pristine and altered samples of Porites sp. consist of aragonite crystals that are poorly cooriented: EBSD scans show identical MUD values: 10 (Fig. 6).", but the coarse domains that suggest some incipient? alteration may escape from being detected by this method; the quality of optical images is too low to assess this (Fig. A11). It is possible that in the Mg^{2+}-rich burial-mimicking diagenetic waters such as used in authors' experiments (Mg^{2+} may also derive from local dissolution of biogenic aragonite) the secondary mineral phase may be the same as original one; in contrast to mollusks whose nacre aragonite tablets have unique morphology, aragonite fibers of corals are morphologically not so distinct from acicular aragonite cements thus distinguishing these two phases can be troublesome (see distinct difference between primary and secondary aragonite in bivalves: Webb et al. 2007 Geology 35: 803–806). Noteworthy,*

the coral skeleton described by the authors as "pristine" e.g., Fig. A12 is full of bioerosion traces (compare Nothdurft et al. 2007: Geochimica et Cosmochimica Acta 71, 5423-5438) which may create additional migration routes of the diagenetic fluids. These problems should be addressed in the text.

We thank the reviewer for the insightful comment. We agree with his remark and have expanded the caption in figure 6 as follows.

*"**Figure 6**. Colour-coded orientation maps with their corresponding pole figures derived from EBSD scans depicting the microstructure and texture of: (a) pristine and (b) altered coral, Porites sp., skeletons. Crystal co-orientation is given by the MUD value. No major changes can be observed between the pristine and the altered skeletal elements regarding co-orientation. <u>However, it cannot be discarded that the diagenetic alteration may have produced variations in the microstructure of Porites sp. which were not detected by EBSD. The low co-orientation of the aragonite crystals in the pristine sample makes it difficult to see major variations after alteration.</u>*

The skeleton of the coral *Porites* sp. is formed by an assembly of differently oriented aragonitic spherulites. EBSD measurements were carried out on highly polished 2D surfaces. In a bidimensional cut of a coral sample, the spherulites may be cut with different oblique orientations. Accordingly, EBSD maps alone (color-coded orientation maps and band contrast maps) may not be sufficient to characterize the microstructure and crystalline orientation of a sample as we may be observing spherulites with different obliquity. We also need (i) texture data derived from pole figures, (ii) the MUD value as a numerical approximation to crystalline co-orientation and (iii) a phase map which may point to possible mineralogical changes. None of the 4 data show differences between the pristine and the altered samples of *Porites* sp. in this work. This occurs despite of the fact that measurements were taken carefully so that the pristine and altered samples could be compared (similar step size, similarly sized regions, pristine and altered samples selected from adjacent regions of the sample...). Moreover, in the work of Casella et al., (2018), the *Porites* sp. skeletons were altered at high temperature (175ºC) and, apart from extensive transformation into calcite, the aragonite crystals underwent significant changes regarding crystal morphology and crystal co-orientation strength relative to their pristine counterparts. The aragonite crystals underwent dramatic microstructural evolution, from spherulites to needles with much higher MUD values. None of these changes were observed in this work neither. This explains why we have stated that we did not observe major differences between pristine and altered samples of *Porites* sp.

To clarify this, we have added the lines below to the third paragraph in page 25 (submitted manuscript version)

Our study shows that the difference in dissolution/decomposition of organics present within the hard tissues is a key parameter in the generation of different volumes of secondary porosity, which adds up to the primary one initially present in the studied biomaterials. Accordingly, the hard tissue of Porites sp. shows a negligible 0.1 wt.% replacement of pristine bioaragonite by abiogenic calcite at 80 °C. Pristine and altered

samples of Porites sp. consist of aragonite crystals that are poorly co-oriented: EBSD scans show identical MUD values: 10 (Fig. 6). In addition, significant differences between the surfaces of pristine and altered Porites sp. samples cannot be detected. AFM images show that pristine and altered Porites sp. surfaces consist of similarly shaped and sized grains, without any detectable signs of mineral unit amalgamation. Statistical analysis of grain size distribution yields no significant difference in grain size between pristine and altered of Porites sp. samples. On the basis of all these observations, we come to the conclusion that Porites sp. skeletons are extremely resistant to long-term hydrothermal alteration at low temperatures. This conclusion is in good agreement with the limited overprint experienced by Porites sp. skeletons in short-term hydrothermal alteration experiments, performed at significantly higher temperatures, 175 °C (Casella et al., 2018; Pederson et al., 2019a). Nevertheless, we cannot completely discard that the microstructure of Porites sp. may have undergone some minor modifications bypassed by the analytical techniques used in this work. The skeleton of Porites sp. was the only one among the samples studied in this work that was collected post-mortem. Therefore, it may have undergone some modifications prior to the beginning of the hydrothermal alteration experiments due to the metabolism of bacteria, algae, boring organisms, etc. Some signs in figure A12 hint to these modifications, which may have created additional routes for the migration of the diagenetic fluids. In any case, the extensive precipitation of abiogenic aragonite that characterizes the second step of the alteration of aragonitic hard tissues was not detected in Porites sp. by any of the techniques used in this work. This contrasts with the results of Casella et al., (2018) where, apart from extensive aragonite transformation into calcite, a reorganization of the aragonite crystals was observed (increase of MUD value, morphological changes in the crystals, etc.). These changes have not been observed in this work.

> ***Remark 3**. AFM images of pristine skeleton of Arctica islandica shell suggest that "aragonite has a slightly rough surface made up of spherical aragonite subunits down to 0.1 µm in diameter (yellow star in Fig. 2)". Similar AFM observations of Haliotis ovina shell suggest that "aragonite prisms have a rough surface composed of up to 0.3 µm aragonite subunits" whereas the aragonitic acicular crystals of Porites sp. are "composed of aragonitic subunits of up to 1 µm".*
>
> *Why there is such significant discrepancy in sizes of observed subunits in comparison to dimensions of subunits documented e.g., by Cuif el al. 2011 " Biominerals and Fossils Through Time"? In mollusks (including different species of Haliotis) the "evidence also suggests that, provided that the presence of irregular rounded nanograins is a feature common to all microstructural units known thus far, this uniformity of morphology of the 10 nm "building block"" (ibidem p. 130). In scleractinian corals, the "skeletal aragonite appears formed of nodular grains with their long axis approximately 100 nm" (ibidem p. 122; see also Benzerara et al. 2011, Ultramicroscopy 111: 1268–1275). In fact, the units illustrated by the authors in Fig. 7 (especially in b and d) are very different from what is typically described as coral skeleton nanostructural organization (moreover these larger subunits e.g., in Fig. 7b are smooth and do not suggest nanogranular organization). Lack of such nanostructural organization is particularly intriguing in experimental samples: removal of organics should result in enhancement of nanograin presence that are thought to be outlined by some organic-rich material. These aspects should be explained in the text.*

We thank the reviewer for the constructive criticism. Images in the works by Cuif et al. (2011) and Benzarara et al. (2011) were taken with higher magnifications than the images in figures 2, 4 and 7, which can explain that biomineral nanoparticulate building blocks appear better defined in the former works. We have reevaluated our estimation of the sizes of building blocks in our samples, both pristine and altered, and have amended the AFM figure captions and the manuscript body accordingly. We are grateful for the correction as it has also allowed us to see that the scale in figure 7 was not right, which has also been amended.

However, we disagree with the reviewer when he states that aragonite nanograins should be more visible in the altered samples. It is true that in the altered skeletons, the removal of the biopolymers should make the aragonitic "building blocks" easier to observe. However, the work has shown with several techniques (EBSD, AFM, grain size evaluation...) that the alteration of the aragonitic microstructures leads to the precipitation of abiogenic aragonite in the secondary porosity formed after the destruction of the organics. By filling this porosity with abiogenic aragonite, the separation between the different nanograins becomes increasingly difficult to distinguish. This is evident in the AFM images of the altered samples (Figures 2e-h; 4e-h), where it can be seen how the aragonitic building blocks appear amalgamated together forming, in some cases, continuous patches (Figures 2f and 4f). The different aragonitic nanograins, therefore, are more difficult to distinguish as the alteration progresses and not the other way round.

> ***Remark 4***. *The scale bars should be normalized in all figures: [Figure 1] Magnifications of all items i.e., a, b, c, d should be the same to be comparable e.g., 50 µm; [Figure 2] Magnifications of all items i.e., a-h (note "f") should be the same to be comparable e.g., 1 µm and 500 nm. Explain magnified region in "f" and provide scale-bar; explain in the caption the yellow star mark, [Figure 3] Magnifications of all items i.e., a, b, c, d should be the same to be comparable e.g., 50 µm [magnification should be the same as in Fig. 1]; [Figure 4] Magnifications of all items i.e., a-h should be the same to be comparable e.g., 1 µm and 500 nm; explain in the caption the yellow star mark ("0.3 µm aragonite subunits (yellow star in Fig. 4b" - found in the main text).*

Scale bars have been normalized in all figures from both, the main text and the appendix figures. Moreover, the captions of Figures 2 and 4 have been amended to explain the meaning of the star marks and magnified regions.

Remarks by Referee 2:

> ***Remark 1***. *Were the animals alive when their biogenic aragonite was sampled, or had the samples experienced some minor marine diagenesis?*

All hard tissues were collected from alive organisms with the exception of the skeleton of *Porites* sp., which was collected *post-mortem* and cannot be discarded that had undergone some degree of minor marine diagenesis. See also the answer to remark 2 by reviewer 1.

> ***Remark 2****. Were the samples weighed in advance, and was the water-rock ratio constant across experiment arms? I wonder about the extreme difference in calcitization of the gastropod between 4 and 6 months of alteration.*

All samples were weighed prior to the beginning of experiments

- The fragments of *Arctica islandica* had an average weight of 0.8 g
- The fragments of *Haliotis ovina* had an average weight of 0.2 g
- The fragments of *Porites* sp. had an average weight of 0.1 g

3 samples of each specimen were inserted in the autoclaves together with 10 ml (10.02 g) of the burial mimicking fluid. As a result, the water/rock ratio was 4.16 for Arctica islandica, 16.66 for Haliotis ovina and 33.33 for Porites sp., respectively. The size of the different animals and the fluid/water ratio was the same as that used by Casella et al., (2018) in high-temperature experiments (175ºC). The same experimental conditions were used in order to allow reliable comparison between the results of both works. The Experimental section of the manuscript has been amended to clearly state this.

Regarding the drastic increase in calcification undergone by *Haliotis ovina* between 4 and 6 months, similar results were observed by Casella et al., (2017) during the alteration of *Arctica islandica* shells at high temperature (175ºC). In this work, the authors observed that the interaction of the shells with the fluid did not produce any replacement of the aragonite by calcite for 4 days. From the fourth day onwards, the replacement proceeded rapidly so that, in just 3 more days, almost all the aragonitic shell had been replaced by abiogenic calcite. The authors argued that this waiting time, which they called "dormant period", would be necessary to build up a sufficiently high ion activity product in the solution to precipitate any calcite. During the dormant period, calcite nucleation would be small, but not zero, due to limited supersaturation. Once supersaturation, calcite nucleation rate and finally, calcite growth rate reached high values the replacement would proceed at a much faster rate. A similar physicochemical evolution can be expected in the system during the long term alteration of *Haliotis ovina* shell (this work). The fact that experiments in this work were conducted at much lower temperature than those in Casella et al., (2017) can explain a much slower alteration kinetics and, consequently, a much longer (several months instead of a few days) dormant period.

> ***Remark 3****. Line 174: was brachiopod shell calcite analyzed in this study? Why is it referred to here?*

No, it was not. The information on the co-orientation of calcite crystals in brachiopod shell is given as an example of fiber or axial texture. In order to avoid any possible misunderstanding, reference to two recent papers (see below) has been added in line 174 (submitted manuscript version).

- Simonet Roda, M., Griesshaber, E., Ziegler, A., Rupp, U., Yin, X., Henkel, D., Häussermann, V., Laudien, J., Brand, U., Eisenhauer, A. and Checa, A.G., 2019. Calcite fibre formation in modern brachiopod shells. *Scientific reports*, *9*(1), pp.1-15.

- Simonet Roda, M., Griesshaber, E., Angiolini, L., Rollion-Bard, C., Harper, E.M., Bitner, M.A., Milner Garcia, S., Ye, F., Henkel, D., Häussermann, V. and Eisenhauer, A., 2022. The architecture of Recent brachiopod shells: diversity of biocrystal and biopolymer assemblages in rhynchonellide, terebratulide, thecideide and craniide shells. *Marine Biology*, *169*(1), pp.1-52.
  Remark 4*. This paper is a valuable contribution towards understanding early burial diagenesis. Do the authors expect significant differences if some alteration first occurred in shallow (low-T) marine diagenetic environments?*

Some degree of biomineral alteration induced by the metabolisms of bacteria, algae, fungi, or different boring organisms can take place even while the organism is alive, more so after its death. Further post-mortem alteration can derive from physicochemical factors (pH, saturation index of the aqueous medium, presence of certain elements, time etc.) The extension of pre-burial alteration undergone by $CaCO_3$ hard tissue can widely vary. Therefore, it is very difficult to anticipate its impact on burial diagenesis and any answer that we could give to this question would be pure speculation. No doubt this is a very interesting topic that needs to be addressed experimentally in future work.

> ***Remark 5****. I appreciate the consistency with other burial diagenesis experiments to facilitate comparison, but why are 100 mM NaCl + 10 mM $MgCl_2$ solutions chosen? This is a lower solute concentration than seawater (465 mM Na^+ + 53 mM Mg^{2+}). Aren't burial formation waters often brinier?*

The reviewer rightly states that burial formation waters are often briner. However, the composition of burial fluids varies widely, and successive exposure of sediments to meteoric and shallow burial fluids is not uncommon. The fluid used in the experiments can be described as a modified seawater. We chose to use the same fluid composition as previous studies (Casella et al., 2017; 2018; Ritter et al., 2017; Pederson et al., 2019a; 2019b; 2020…) to facilitate comparison. Moreover, although it is unlikely that the use of fluids with higher NaCl content would have any major impact on the kinetics of diagenetic alteration, higher $MgCl_2$ contents may strongly inhibit aragonite recrystallization into calcite, making necessary even longer experimental runs to observe the subtle changes characteristic of early diagenesis.