egusphere-2023-631_Review_JR_ Julien Richirt

The study investigates the impact of various seawater sulphate concentrations on carbonate associated sulphate (CAS) content and sulphur isotopic composition of a benthic foraminifera morphotype. To do so, the authors cultured for ~30 days specimens of *Rosalina*-like foraminifera from 2 strains coming from Mediterranean and Atlantic French coast in different media where the [SO_4^{-2}] varied between 0 and 180 mM. Acclimatised for a long period of time before the experiment, CAS content and sulphur isotopic composition of their shell was measured after exposure to various [SO_4^{-2}] concentrations. During the experiment, the abundance of living individuals was recorded in the different experimental conditions to evaluate their tolerance to the different [SO_4^{-2}] in their surrounding environment. The general aim of the study is to explore the possibility to use foraminifera shell composition as a proxy for paleoenvironmental reconstructions of [SO_4^{-2}] in seawater.

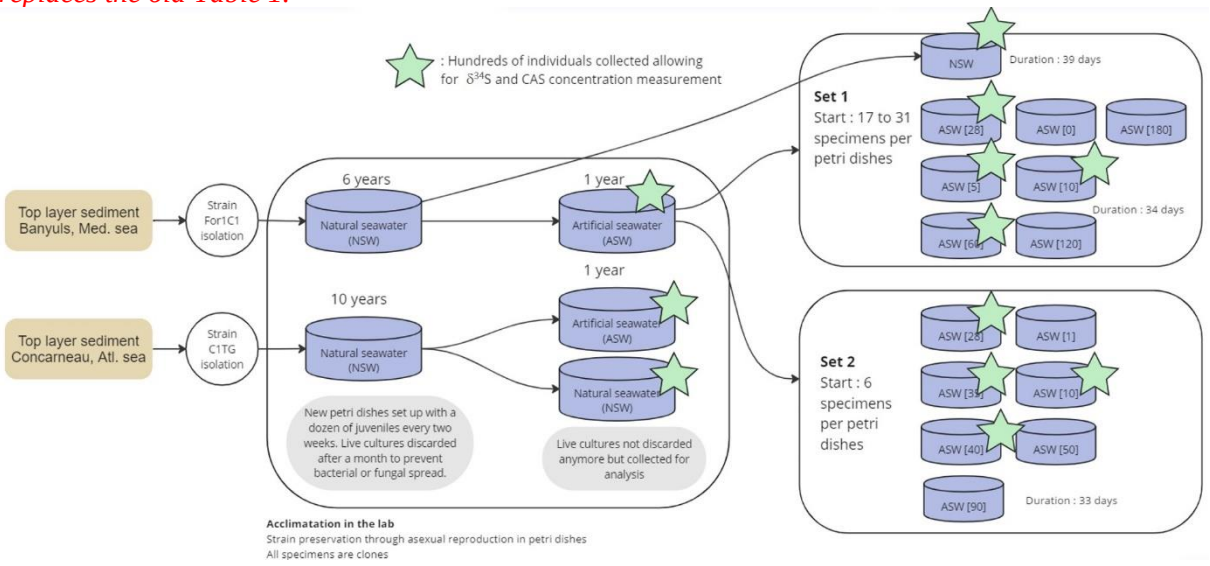
I found the manuscript interesting to read, and I think experimental approaches of this kind, while very difficult to realise in practice, are necessary to better understand how Foraminifera respond to their environment. I think this work can be very useful for the community regarding potential future [SO_4^{-2}] proxy calibrations using foraminifer's test, and more generally concerning S-cycle understanding. This manuscript in the scope of the journal and is suited for publication in Biogeosciences.

However, I had some difficulties to comprehend certain parts of the manuscript and I sometimes did not understand fully interpretations and/or conclusions made by the authors. In some places I think that some sentences should be rephrased and that some terms are either misused or too vague. I list below major points that I consider important to be addressed by the authors before publication.

We thank Dr. Richirt for this supportive comment. We have worked to reply to all of his questions and gave a lot of attention to rephrasing sentences that could have been considered as unsufficiently precise. We believe that the manuscript benefitted from his comments and suggestions and we hope that we have successfully addressed his concerns.

1) The experimental protocol contains a lot of steps, and it took me a while before understanding it correctly. I think that the manuscript would highly benefit of either a workflow graph replacing table 1, or another possibility would be to indicate more details in table 1, such as: sampling year for each strain, number of years maintained in NSW, time of acclimation, number of specimens per condition, duration of each experiment, days of abundance counting… All these informations are available in the plain text and/or in other tables/graphs, but I think that to put everything in 1 graph (or table) would really make it easier for the reader to grasp all the efforts the authors put in the experimental settings.

We have followed this suggestion and now a new Figure 1 showing the experimental protocol workflow replaces the old Table 1.



2) On results & interpretations regarding foraminiferal abundances in experiments.

a. At the end of section 3.1, you compare abundances of individuals in the different media at the end of the experiment. Line 209-210, you state that the highest abundances were found for ASW28, NSW, ASW10 and ASW35. While the statement in absolutely correct, I would also consider in this list ASW5 (425 ind.) which is very close to the condition ASW35 (470 ind.).

We have amended the text as suggested and added the ASW5 configuration to the list:" Overall, the highest numbers of individuals at the end of the experiment were obtained in the ASW[28], NSW (Banyuls), ASW[5], ASW[10] and ASW[35] media (Fig. 5 = old Fig. 4)."

b. At the end of the same paragraph (lines 210-215), you try to explain the difference you observe between ASW10 in set1 and set2. I find the explanation unclear and probably unnecessary. For instance, what do you mean by "more carefully" about the individual selection before the experiment? Another reason why reproduction rate could be different between the 2 sets is that they just have more space in the Petri to multiply more efficiently, or less competition for food (if you put the same amount in all Petri)? Finally, abundances for ASW10 in both sets are of the same order of magnitude. Your graphical representation (log scale, fig. 4) is suitable to visualise this, the conditions 10mM from the 2 sets show relatively similar values regarding the sampling date. However, what must be discussed here (which is done in supplementary material appendix C) is the rather surprising low abundances of condition 28.2 set 2 compared with condition 28.2 set 1. I suggest mentioning it in section 3.1 instead of mentioning it only in appendix.

In accordance with this suggestion, we have made the following changes to the text:"Two media configurations, ASW[10] and ASW[28], from set 1 experiments were replicated in set 2 experiments. If the abundances for condition ASW[10] are of the same order of magnitude in both sets, the abundances for condition ASW[28] are much lower in set 2 compared to set 1. This was related to the reproduction rate in set 2, which slowed down drastically after 15 days. This decrease can be explained by a microbial bloom in the media that was observed in no other media (Appendix, Fig. C1). The microbial spread could not be reduced by the weekly water change, and any transfer and rinsing of foraminifera or antibiotic treatment would have constituted an additional experimental modification. We thus kept counting foraminifera and sampling seawater, but did not take into account any results collected in that media after day 15."

3) On results & interpretations regarding $SO_4^{2-}/CaCO_3$ ratio.

a. In section 3.3 about CAS concentration, on lines 226-227 you list the conditions for which enough tests could be collected for CAS analyses. However, I miss ASW50 (117 ind.) and ASW1 (161 ind.) both having more specimens than ASW60 (108). Why couldn't you perform analyses for these 2 conditions?

We have rephrased the sentence by deleting "for each medium when enough tests could be collected for analyses" and adding " as the other samples were unfortunately lost during the manipulations or were below the detection limits."

b. I might have misunderstood here, but in the same section on lines 229-232, you state that there is a threshold at 14000 ppm $SO_4^{-2}/CaCO_3$ for the 40mM condition. However, in table 4 I see a value of 11600 ppm for this condition. Figure 4 is consistent with table 4 values. Same problem for ASW5 condition for which it is 3320 ppm in your text (l. 229) but 2740 ppm in your table 4 and fig. 6. Please clarify or correct these values, since it is a crucial point for your further discussion.

There was an error in the annotation of the values in Table and Figure 4 (now Figure 5). We have now homogenized the CAS values presented in the text, tables and figures. We have reworded the paragraph to make it clearer, in agreement with the reviewer, that there is a plateau from 28 mM to 60 mM and probably a threshold effect from 28 mM, given the error bars of the values.

4) You use the term "physiology" in the abstract and in section 4.1 and I find the term not suitable to use in the context of this study. It is very confusing for me here because you find living individuals in all conditions at the end of your experiments. This indicates that individuals survive in all conditions, suggesting that they can sustain their physiological activity (even partially) in all conditions. However,

as your results point out, their capacity to reproduce seems to be dependent on seawater [SO_4^{-2}]. I suggest being more specific in the text and replace "physiology" with what is actually evaluated (reproduction rate/pseudopodial activity/survival…).
For instance, on line 265 (first occurrence in the text excluding the abstract):
"Thus, our results suggest that foraminifera can sustain their physiological activity only within a certain range of [SO_4^{2-}] …."
Could be replaced by:
"Thus, our results suggest that foraminifera can reproduce only within a certain range of [SO_4^{2-}] …."

We have followed this suggestion and replaced the term "physiology" with reproduction, pseudopodial activity, etc.
However, we added in the discussions, paragraph 4.1 "In particular high seawater [SO42-] (> 35 Mm) inhibit the foraminiferal proliferation by an undetermined toxic effect on the cellular physiology".
The reduction in reproduction implies that cellular physiology has been affected, although we do not yet know the exact mechanisms and effects.

I would like to inform the editor that I am not comfortable with the evaluation of the isotopic fractionation methods and result interpretations since it is out of my field of expertise. For this reason, I cannot fully assess the relevance of this part in the manuscript.
I think that some points, especially data and conclusion drawn from them, must be clarified/corrected before publication. Consequently, I recommend major revisions for this manuscript.
I require my name to be attached to this review, since it is not double anonymised.
Julien Richirt

Detailed comments and suggestions (associated with annotated pdf file)

Abstract
Line 27: please replace the term "physiology" by a more specific term.
Done

Introduction
Line 62: "interrogates".
Done

Line 65: "over a 180-fold range of seawater …". This is unclear, please specify the range, from 0 to 180.
Done

Materials and Methods
Line 85: Please specify if 90mm is the diameter or radius of the Petri dish you used.
Done

Line 97: please specify why it is more suited for geochemical studies.
There was a shortcut in the logic of the sentence, we have rewritten this part as follows:
" Live algae can have a major impact on the seawater carbonate chemistry system by reproducing and consuming CO2 through photosynthesis. As freshwater algae, the Chlorogonium cells died immediately in seawater, without undergoing lysis. This prevents those not eaten by foraminifera from spreading and/or being metabolically active and thus they do not influence the seawater chemistry conditions within the Petri dishes. The use of live freshwater instead of seawater algae to feed foraminifera is therefore an innovative approach that is particularly suited to long term culture experiments for the calibration of foraminiferal geochemical proxies, where seawater chemical conditions must be kept under control".

Line 100: what do you mean by "every other week"?

"Every other week" means "every two weeks".

Line 112: Is it true for train C1Tg which was not cultured in different sulphate concentrations?
To clarify this question, we now add the following text: "The C1Tg strain was only used for [SO_4^{2-}] and $\delta^{34}S$ composition measurements of specimens from media in ASW or NSW at the current seawater average [SO_4^{2-}] of 28 mM, whereas the For1C1 strain was also used for [SO_4^{2-}] and $\delta^{34}S$ composition measurements of specimens from media with different [SO_4^{2-}]".

Line 120: Table 1 lack some important informations that deserve to be in the main text and not in supplementary, such as number of individuals in each conditions.
We have followed this suggestion and have now moved Table B1 from supplementary to the main text as new Table 1, and the sentence now refers to new Figure 1 (Experimental protocol workflow graph) and new Table 1 (Weekly number of accumulated live individuals for each medium at different SO_4^{-2} concentrations).

Line 127: What do you consider as a large population? When did you do this in the experiment course and for which conditions?
We now specify in the text "For populations of more than approximately 300 individuals, as obtained in media with concentration ranging from 5 to 35mM sulfate"

Line 128: "superpopulation" this term means something else, I guess you mean overpopulation?
Done

Line 128: "chlorogonium" should be in italic and capitalised.
Done

Line 129: "petri" sometimes capitalised sometimes not, please homogenise.
We now homogenized all in capital

Line 144-145: incomplete sentence
*We have now completed the sentence as follow: "*The CO_2 and the He mix was then sampled with an autosampler and sent to a Dual Inlet FinniganTM DeltaPlus XP isotope ratio mass spectrometer"

Results
Line 192: what do you mean by low cell density?
We now specify: "where cells do not compete for food" and " less than approximately 300 individuals per Petri dishes" in our case

Line 200: you could rephrase "number of individuals produced by the same cell" by "number of juveniles produced by individual". *Done*

Lines 209-215: see my general comment 2).
We have amended this part in line with comment 2.

Line 219: in table 2 for ASW[5] I see ±0.3, not ±0.2
We have now corrected and replaced 0.2 with 0.3

Lines 226-234: see my general comment 3).
We have now corrected the CAS values in Fig. 7 (old Fig. 6) and Table 4 according to the text.
Following JR's suggestion in the annotated pdf of the manuscript, we have now standardised that the threshold effect in foraminiferal CAS is reached at about 28mM seawater sulfate concentration.

Lines 240: if you state that a difference is significant between 2 values, you should provide the statistical procedure you applied.

"NSW $\delta^{34}S$ composition was measured before (21.1±0.2‰) and 7 days after adding the algae (19.9±0.2‰). There was a significant difference between the two values."
We have now added in the last sentence "beyond error bars"

Discussion
Line 262: "dissolved sulfate in seawater is necessary for cellular activity in foraminifera". Out of curiosity, do you have any idea why they need sulfate from their surrounding water?

Sulfur is essential for life, and sulfate in seawater, which is very abundant, can be an important source of sulfur for marine organisms. In the sentence: "In this experiment, dissolved sulfate and food were the only sources of sulfur", we added: "which is essential for life".

Lines 265-266: see general comment 4). What I see on fig 4 is that, excluding the first reproduction event, condition 90 of set 2 is relatively similar to conditions 0, 120, 180 of set 2. Could it be that individuals might tolerate 90mM condition for 1 week and then stop any reproductive activity?
In line with this comment, we have now added to the text "Individuals appear to tolerate these extreme conditions for only the first week and then cease all reproductive activity".

Line 267: "In this experiment", I guess you mean the whole experiment, both sets? Please specify.
We have now specified as both experiment sets

Line 267: "weekly accumulated" what does that mean? aren't experiments running for about 1 month? Or is it the number of new living individuals added for each week? In this case what weeks are you looking at? Did you consider absolute abundances or proportion increase?
For "weekly accumulated" individuals, we meant the number of new living individuals accumulating each week, incremented by reproduction, and we consider as absolute abundances (see table 2, ex table B2). In this part we mean at the end of each set of experiments. We have now better specified "the high number of accumulated the high number of accumulated live individuals incremented by reproduction at the end of set 1 and set 2 experiments"

Line 268: "in both artificial and natural seawater" True only for set 1. If you consider condition 10mM of set2 (1014 ind. at 33 days) and NSW of set1 (732 ind. at 34 day), then this conclusion is incorrect. If you consider the number of individuals added week after week, then condition 10mM of set 1 also show a growth of about 300 ind. between 18/04 and 25/04, but I have the impression that I did not really get what you mean here.
We have now corrected this part according this comment. As also presented in the results we have now wrote that individuals of the For1C1 strain appear to be well adaptable, beyond the modern oceanic [SO42-] (28.2 mM) to a range of seawater [SO42-] from 5 to 35 mM, as shown by the high number of accumulated live individuals at the end of set 1 and set 2 experiments

Line 268: "suggesting that these species are highly adapted to their actual environment". I assume that by actual you mean modern. This suggestion is difficult to believe because the first part of the sentence is unclear.
We have now changed this part in accordance with the previous comment

Lines 269-270 and 274: please change the term physiology.
We have changed the term physiology as suggested by the reviewer.

Line 295: This is true for the first week, after that the number of individuals does not seem to grow much more. They might only tolerate short exposure to such high sulphate in their environment?
Following this comment, we have now added "However, their reproduction is limited to the first week, which shows that they could only tolerate brief exposure to such a high level of sulfates in their environment."

Line 305: please correct the author name in reference, also on lines 321 and 365.

Done

Lines 308: remove comma.
Done

Line 319: see general comment 3). Seeing fig 4, the moment they stop to incorporate more sulphate is about 30mM, not 40mM.
The reviewer is probably referring to Figure 6 and not Figure 4. We have corrected the text to replace "40 mM" with "above 28 mM".

Lines 342: I might be wrong here, but is not this organic sulphur source from algae? This value is 7 in fig. 7, table B2, and main text one line 241.
It is actually related to the value of algae, we corrected and replaced it with the value 7.

Lines 359-360: the CAS concentration reaches a plateau at about 30mM, not 40mM. see general comment 3).
We have corrected this part, also taking into account the comments noted in the pdf, as follows: "Our results show that benthic foraminifera (Rosalinidae) incorporate CAS in their test proportionally to the [SO_4^{2-}] in seawater, confirming previous experiments on planktic foraminifers that foraminiferal CAS can serve as a proxy for variations of both $\delta^{34}S_{CAS}$ and [SO42-] in seawater (Paris et al., 2014). However, they also highlight that above the seawater [SO_4^{2-}] of 28 mM, it is not possible to confidently determine the seawater [SO_4^{2-}] using foraminiferal CAS, as the previous linear correlation no longer holds."

Lines 368: "can affect the production of carbonate by affecting the biology of certain organisms". Too vague and too strong statement. I guess you mean reproduction rate/survival of your strain of benthic foraminifera? I recommend to at least change "can" for "could" to weaken this rather too strong statement!
We corrected according the suggestion of reviewer and we wrote as follow : "could affect the production of carbonate by affecting the reproduction rate/survival of certain organisms, as in the case of the benthic foraminifera studied in this work."

Lines 370-374: very difficult to understand this sentence, simplify or make 2 sentences.
Following this comment, we have simplified and made 2 sentences as follows: "This work illustrated *how variations in seawater composition can have a dual effect on biomineralizing organisms. Conditions that inhibit calcite formation such as increases in marine concentrations of Mg2+ or SO42- , could have chemical "abiotic" effects on carbonates formation but could also affect biological processes involved in biomineralization.*"

Conclusion
Lines 379: "…foraminiferal biology…" please be more specific
We corrected and replaced "foraminiferal biology" with "foraminiferal reproduction"

Lines 381: "concentrations above 90mM becomes toxic and lethal". This is not what your data are suggesting! You have living individuals in all your conditions (fig 4, tables B1 and B2), and their abundance is most of the time very close to the number of individuals you initially put in the Petri dish! This rather suggest that they can survive these high sulphate concentration but cannot reproduce!
We have corrected according this comment: "Sulfate from seawater is necessary for the cellular activity of foraminifera, but at concentrations equal and above 90 mM it becomes toxic to them, as evidenced by cellular inactivity and reproductive arrest"

Figures & Tables

Fig 4. The legend for the line color of set1. Could you reorganise the display by sorting it by concentration such as on the right panel (set2).

We have followed this suggestion and now in the Figure 5 (old figure 4) the line color of set 1 is reorganized as those of set 1.

Table 1. see general comment 1).

We have followed this suggestion and now a new Figure 1 showing the experimental protocol workflow replaces Table 1.

Table 4. for the strain C1Tg, SO4/CaCO3 values, I am surprised by the rather large variability of these values for NSW and ASW28 compared to the other strain. Did you used these data somewhere in the manuscript or other figures?

There is indeed a reproducible difference in the concentration of CAS in one species to another, which is yet another argument in favor of species-specific calibration, as stated line 410.

"The use of CAS concentration as a marine [SO42-] record is thus still promising, despite the limitation discussed above, but will require calibration on various types of carbonates and species" .

We thus do not compare CAS concentration variations from one specie to the other in this paper.

Table B1. I used these 2 table quite a lot during the review, I think showing these data in the main text might be useful for the reader. This is only a suggestion.

We followed this suggestion and we moved the table B1 in the main text and named now as table 2

Table B2. Please add unit

Now table B1, we added ‰

Appendix C. see general comment 2). The part about the microbial growth in the ASW28 condition set 2 is important to explain your results in my opinion and I would mention this in the main text rather than in appendix.

We followed this suggestion