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.

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.

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.).
    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.

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?
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.

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 $[SO_4^{2-}]$ …."

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.

Introduction

Line 62: “interrogates”.

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

Materials and Methods

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

Line 97: please specify why it is more suited for geochemical studies.

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

Line 112: Is it true for train C1Tg which was not cultured in different sulphate concentrations?

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.

Line 127: What do you consider as a large population? When did you do this in the experiment course and for which conditions?

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

Line 128: “chlorogonium” should be in italic and capitalised.

Line 129: “petri” sometimes capitalised sometimes not, please homogenise.

Line 144-145: incomplete sentence

Results

Line 192: what do you mean by low cell density?

Line 200: you could rephrase “number of individuals produced by the same cell” by “number of juveniles produced by individual”.

Lines 209-215: see my general comment 2).

Line 219: in table 2 for ASW[5] I see ±0.3, not ±0.2

Lines 226-234: see my general comment 3).

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

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?

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?

Line 267: “In this experiment”, I guess you mean the whole experiment, both sets? Please specify.

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?

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.

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.

Lines 269-270 and 274: please change the term physiology.

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?

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

Lines 308: remove comma.

Line 319: see general comment 3). Seeing fig 4, the moment they stop to incorporate more sulphate is about 30mM, not 40mM.

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.

Lines 359-360: the CAS concentration reaches a plateau at about 30mM, not 40mM. see general comment 3).

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!

Lines 370-374: very difficult to understand this sentence, simplify or make 2 sentences.

Conclusion

Lines 379: “…foraminiferal biology…” please be more specific

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!

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).

Table 1. see general comment 1).

Table 4. for the strain C1Tg, $SO_4/CaCO_3$ 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?

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.

Table B2. Please add unit

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.