

Answers point by point to reviewers

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 $[\text{SO}_4^{2-}]$ 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 $[\text{SO}_4^{2-}]$ concentrations. During the experiment, the abundance of living individuals was recorded in the different experimental conditions to evaluate their tolerance to the different $[\text{SO}_4^{2-}]$ 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 $[\text{SO}_4^{2-}]$ in seawater.

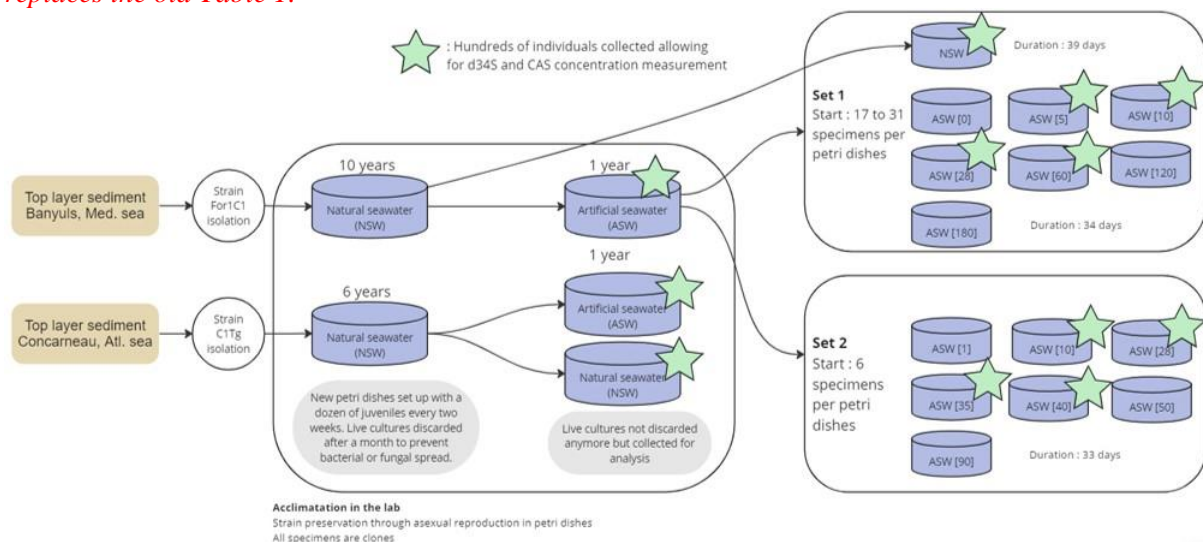
I found the manuscript interesting to read, and I think experimental approaches of this kind, while very difficult to realise in practice, are necessary to better understand how Foraminifera respond to their environment. I think this work can be very useful for the community regarding potential future $[\text{SO}_4^{2-}]$ proxy calibrations using foraminifer's test, and more generally concerning S-cycle understanding. This manuscript is 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 insufficiently 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 is 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 $\text{SO}_4^{2-}/\text{CaCO}_3$ 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 $\text{SO}_4^{2-}/\text{CaCO}_3$ 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 $[SO_4^{2-}]$ (> 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 CO_2 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 [SO₄²⁻] and δ³⁴S composition measurements of specimens from media in ASW or NSW at the current seawater average [SO₄²⁻] of 28 mM, whereas the For1C1 strain was also used for [SO₄²⁻] and δ³⁴S composition measurements of specimens from media with different [SO₄²⁻].”

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 SO₄²⁻ 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 CO₂ 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 $\delta^{34}\text{S}$ composition was measured before ($21.1\pm 0.2\%$) and 7 days after adding the algae ($19.9\pm 0.2\%$). 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 $[SO_4^{2-}]$ 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 Mg^{2+} or SO_4^{2-} , 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

egusphere-2023-631_Review 2_E_David_Evans

The manuscript 'Impact of seawater sulfate concentration on sulfur concentration and isotopic composition in calcite of two cultured benthic foraminifera' by Thaler *et al.* presents laboratory culture experiments of a strain of Rosalinid foraminifera grown under different seawater sulphate concentrations and sulphur isotopic compositions. The rationale is that foraminiferal carbonate associated sulphate (CAS), i.e., sulphate that is presumably incorporated as an impurity into the mineral lattice, may be an archive of past changes in the seawater sulphate concentration and isotopic composition. To this end, the authors have performed a set of carefully-conducted and well-described experiments covering a wide range in [SO₄²⁻] and δ³⁴S. The results show that, up to a point, the foraminifer CAS concentration is proportional to that in seawater, and that δ³⁴S_{shell} is related to δ³⁴S_{seawater} with a slope of 1 but an offset of 1.4-1.6 ‰. Together, these results provide promising indication that foraminiferal CAS is likely to be a useful archive of past changes in the sulphur cycle, while highlighting the need for calibration datasets to identify species-specific vital effects.

I have a few main comments, related to the possible impact of seawater carbonate chemistry on some of the results and whether or not the data presented here really enable us to say something about the organisms' ability to regulate sulphate within the cell or at the biomineralisation site via active transport. While I suggest rephrasing parts of the discussion with this in mind, the manuscript is interesting and presents an important dataset, which I look forward to seeing published.

We thank Dr. Evans for this encouraging comment and took into account all of his comments as we detail afterwards. We hope that we have addressed all his concerns and thank him again for his review that contributed to improve our manuscript.

Main comments

1. As the authors acknowledge, previous work has identified the seawater SO₄²⁻/CO₃²⁻ ratio as being a likely key control on S/Ca, as SO₄²⁻ probably competes for the anion position in calcite. Given this, experimental work aiming to understand S incorporation must have excellent carbonate chemistry control, which is largely the case here (pH and DIC were measured and were broadly held constant). It does not appear that changes/variations in seawater carbonate chemistry within and between experiments is a major issue for this study, but I would suggest that the authors calculate SO₄²⁻/CO₃²⁻ for each experiment, and additionally (or alternatively) plot the results (Fig. 6) against this parameter. For example, the DIC in experiment ASW[60] was ~20% higher than in ASW[40]/ASW[50], which may explain the lower S/Ca ratio of this experiment.

To reply to this useful comment, we have performed a computational geochemical modelling using the software JCHESS, available in the Appendix, taking into account our media configurations where we had CAS data. This model shows that the CAS concentration follows linearly the seawater SO₄²⁻/CO₃²⁻ or the seawater sulfate concentration, though the linearity of the relationship is different when we consider only free sulfate instead of total sulfate (free and in complexes) in the solutions.

However, formation of complexes in solution do not appear to explain the disappearance of the linear relationship between CAS and sulfate concentration in solution at high sulfate concentrations (see new figure 8).

2. In several places the manuscript contains inferences about biomineralisation which are very interesting but for which there is arguably little evidence based on the data presented here. For example, in Sec. 4.2, the possibility that foraminifera can regulate the biomineralisation site [SO₄²⁻] or actively maintain a constant SO₄²⁻/CO₃²⁻ is mentioned. Both seem unlikely to me, although it is clearly stated that these are hypotheses, which is of course fine. However, these hypotheses appear in more certain terms elsewhere (e.g. the abstract 'highlighting the extent of control on the precipitation fluid chemistry...' (line 26) which should be removed or qualified, and lines 30, 44, 301 (even within the framework of hypothesising, I don't think 'probably indicates' is accurate), lines 370-375, 381). All of these sentences/sections should be rephrased more cautiously in my view.

We understand the reviewer's concern. As a result, all the sentences about the implications on biomineralisation have been rephrased more cautiously. :

In the abstract we replaced :

" Foraminiferal CAS concentration increased proportionally with $[SO_4^{2-}]$ concentration from 5 mM up to a threshold value of 40 mM, highlighting the extent of control on the precipitation fluid chemistry that foraminifera exert on the carbonate precipitation loci."

By :

"Foraminiferal CAS concentration increased proportionally with $[SO_4^{2-}]$ concentration from 5 mM up to 28 mM, and then showed a plateau from 28 to 60 mM. The existence of a threshold at 28 mM is interpreted as the result of a control on the precipitation fluid chemistry that foraminifera exert on the carbonate precipitation loci. However, at high seawater sulfate concentrations (> 40 mM) the formation of sulfate complexes with other cations, may partially contribute to the non-linearity of the CAS concentration in foraminiferal tests at high increases in $[SO_4^{2-}]$."

And in the discussion we now state :

"i) Foraminifera may be able to regulate $[SO_4^{2-}]$ at the site of calcification (SOC) during calcite precipitation through active transmembrane transport, removing excess sulfate and lowering it in the precipitating fluid, enabling calcite nucleation and precipitation, as sulfate in high concentration inhibits calcite precipitation and makes it more soluble (Busenberg and Plummer, 1985; Bots et al., 2011; Barkan et al., 2020). In fact, under our experimental conditions the amount of CAS incorporated in foraminiferal calcite correlates with seawater SO_4^{2-} concentration, from 5 up to a plateau that starts at 28 mM. The mere fact that calcite precipitates therefore suggests that sulfate is at least partially removed from the precipitating fluid, altering the local SO_4^{2-} concentration. The correlation suggests that this removal is partial and, to some extent, proportional to the concentration of SO_4^{2-} in seawater.

"

3. Adaptation. The importance of acclimatisation and the benefit of working with benthic foraminifera is highlighted in a couple of places in the manuscript (e.g. line 68), but it was not quite clear to me whether only specimens that grew entirely under experimental conditions were selected for analysis, and to what extent reproduction can be considered adaptation. Was the original population/were empty shells following reproduction removed before geochemical characterisation? Please clarify. If not, then the point about acclimatisation to experimental conditions doesn't stand.

We clarified by adding the sentence: "Only live individuals (not empty shells that were discarded in previous water changes) that had fully grown under the experimental conditions were selected for analysis."

We added also in the paragraph 2.2 "Collection and rinsing procedure of the tests for geochemical analyses" that "all live individuals of the strain For1C1, as specified above, those still attached to the substrate" from each Petri dish were recovered for geochemical analyses.

4. Complexation is mentioned in the discussion, and I agree that it would be helpful to calculate speciation in these experiments. Indeed, doing so (using phreeqc; see figure) highlights that i) the experimental design means that Ω_{calcite} decreased with increasing $[SO_4^{2-}]$, and ii) that at the extreme high end of the $[SO_4^{2-}]$ studied here, the seawater was possibly saturated with respect to gypsum/celestite. Again, I doubt this impacts any of the main conclusions, but it would be good to include a more thorough discussion of the topic rather than talking in very general terms (lines 311-314). For example, perhaps the decreasing Ω_{calcite} contributed to the lower growth/survival rates of

foraminifera grown at higher [SO₄²⁻], given that Ω_{calcite} approximately halves between the lowest and highest [SO₄²⁻] experiments.

Finally, given that some of the experimental seawaters used here are close to being oversaturated with respect to gypsum and celestite, was any inorganic precipitation observed in the cultures? Gypsum precipitation seems unlikely, but e.g. if [S_{rsw}] was a little higher than the assumed/target value, then this may have been an issue in the higher [SO₄²⁻] experiments.

[Phreeqc calculations using the solution compositions given in Sec. 2.1.2, pH = 8.2, DIC = 4 mM, and the pitzer database. The Matlab code used to produce the figure is given on page 4.]

We did not observe any precipitation of gypsum/celestite in the culture media at the extreme high [SO₄²⁻], but the formation of complexes and its impact on CAS concentration is a very important point. We have now added a geochemical model in the appendix, which takes into account our media configurations where we had CAS data. (This new Appendix D is available at the end of this paragraph).

We have also added in the discussion that this model shows that the model CAS concentration follows linearly the seawater SO₄²⁻/CO₃²⁻ and CaSO₄/CaHCO₃ concentrations, which in turn depend mainly on the [SO₄²⁻] in solution, with a dip between 40 and 60mM, likely related to the formation of complexes. At sulfate seawater concentrations > 40 mM sulfate might complex more easily with other cations (Ca²⁺, K⁺, Mg²⁺, Na⁺, Sr²⁺) and such complexes cannot be effectively incorporated into the calcite lattice structure.

Regarding the effect of Ω_{calcite} on foraminifers, at low sulfate concentration, where Ω_{calcite} increases, we observe less reproduction and thus less calcite formation. We hence believe that calcite formation in our system is rather controlled by foraminifers biological state (that seems related to solution sulfate concentration in such extreme variations) and to a much lesser extent to Ω_{calcite} in solution.

6.3 Appendix D

815 Geochemical modeling Parameters

The relative abundances of Ca²⁺, SO₄²⁻, CaSO_{4(aq)}, MgSO_{4(aq)}, NaSO_{4(aq)}, CaHCO₃, CO₃²⁻, HCO₃⁻ and CO_{2(aq)} in solution were computed with the geochemical code JCHESS (Van der Lee, 1998). ASW composition from Kester et al., 1967 and experimental temperature (20 C) were chosen as input parameters assuming a closed system with no gas-solution exchange, and Cl, Na and SO₄²⁻ were modified as they were in each media of the experiment (Base input in the additional excel table). All DIC is provided as HCO₃⁻ to the model.

820

Given that pH was adjusted by NaOH addition, this was reproduced in the model, by adding Na⁺ and OH⁻ (in the same concentration) until reaching pH 8.2.

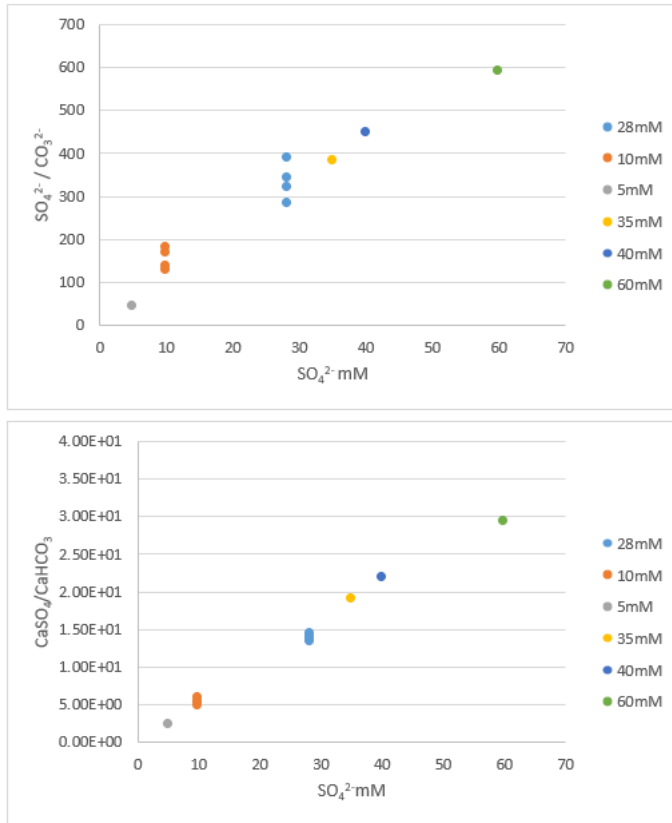
From this starting point, HCO₃⁻ was adjusted to the measured DIC value, and OH⁻ was adjusted to match the measured pH value (Adjusted input, and measured values in the additional excel table). No additional Na⁺ was added despite the slight electrical imbalance generated, as Na⁺ can form complexes with SO₄²⁻ and no Na⁺ was provided to the media after pH has been adjusted. The output data considered are Ca²⁺, all free and complexed SO₄²⁻ species, free DIC species and CaHCO₃⁻. The sole DIC species present as a complex that was extracted is CaHCO₃⁻, because it is the major complex. It is also a species that has been hypothesised to be potentially incorporated into calcite, as CaSO₄ could be.

825

830 Geochemical modelling results

The SO₄²⁻/CO₃²⁻ concentration increases linearly to a slight inflection point at 60mM, linked to complexes formation. Nevertheless, we do not observe a plateau from 28mM onwards, which could have explained a constant incorporation of SO₄²⁻ in calcite beyond 28mM. Similarly, assuming that SO₄²⁻ incorporation into calcite takes place from CaSO₄, although an inflection of the CaSO₄/CaHCO₃ ratio is observable from 40mM, no plateau is observed. These results show that the incorporation of sulfate into the calcite of the foraminifers in our experiments, which plateau above 28mM, cannot be explained by the formation of complexes in seawater. Alternatively, CAS concentration is not a good recorder of either the SO₄²⁻/CO₃²⁻ ratio or the CaSO₄/CaHCO₃ ratio above a concentration of 28mM.

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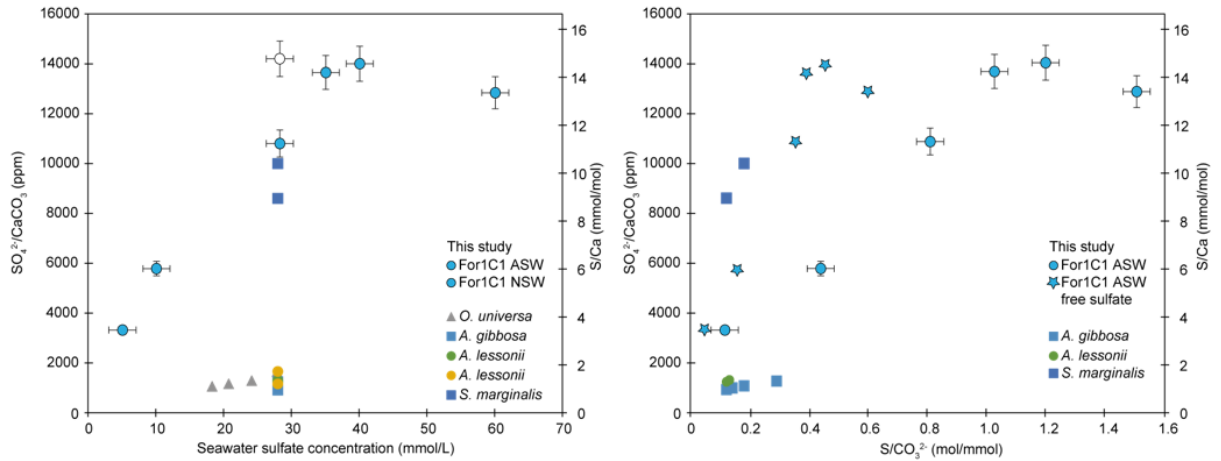


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Figure D1. Geochemical modeling results showing $\text{SO}_4^{2-}/\text{CO}_3^{2-}$ ratio and $\text{CaSO}_4/\text{CaHCO}_3$ ratio in solution as a function of total sulfate concentration in solution. Each point corresponds to a different computational run, for samples where DIC and pH were measured, and were used as constraints to the model. Both ratios increase linearly to a slight inflection point at 60mM, but no plateau is seen between 28mM and 60mM.

5. There isn't a lot of foraminifera S/Ca data out there, so it would be a nice addition to the manuscript (and not too much work) to compare directly to previous studies, especially that of van Dijk et al. [2017].

We have followed this suggestion by adding data from previous studies to Figure 7 and new Table B2 in the Appendix. We have also included a comparison of our data with those published in the literature in the introduction to section 4.2 of the Discussion.



725 **Figure 7. Left panel: $\text{SO}_4^{2-}/\text{CaCO}_3$ and S/Ca ratios on tests of the For1C1 strain at the end of SET1 and SET2 experiments, as a function of seawater $[\text{SO}_4^{2-}]$ (5, 10, 28.2, 35, 40 and 60 mM). Right panel: $\text{SO}_4^{2-}/\text{CaCO}_3$ and S/Ca ratios on tests of the For1C1 strain at the end of SET1 and SET2 experiments, as a function of seawater $\text{S}/\text{CO}_3^{2-}$. For our experimental results, we report the values using both S as the sum of free and complexed sulfate based on our model results (circles), and as only free sulfate (stars). Each measurement has been performed on a pool of hundred to several hundreds of specimens. Values are compared to other culture experiments of foraminifera targeting specifically the CAS content of the tests (Paris et al., 2014; van Dijk et al., 2017; 2019), and, when available, $\text{S}/\text{CO}_3^{2-}$ as well.**

730 See Appendix B, Table B2 for details.

Minor comments

1. Line 23. Consider clarifying in the abstract why this range is different than on line 21 (the reason is given later, but to avoid confusion).

We clarified that while the benthic foraminifera were cultured under controlled conditions with seawater $[\text{SO}_4^{2-}]$ ranging from 0 mM to 180 mM (line 21), we measured CAS and $d_{34}\text{S}$ (line 23) on samples from “a selection of culture media” where $[\text{SO}_4^{2-}]$ varied from 5 to 60 mM.

And line 266 it is now specified: “CAS concentration in foraminiferal calcite was performed for the media ASW[5], ASW[10], ASW[28], ASW[35], ASW[40] and ASW[60], as the other samples were unfortunately lost during the manipulations or were below the detection limits”.

2. Line 36. Clarify that this is the case at higher (room) temperature.

We clarified adding in the sentence “at room temperature”

3. Line 124. I didn't understand – it is stated that the second set was ‘designed to extend our concentration range’, but the range is narrower than in the first set.

We have corrected this sentence (see also the reply to Julien Richirt's comments) as follows “designed to refine the concentration step between 0 and 90 mM”.

4. Line 149. Table 3 alternatively states $\pm 4\%$.

It was a mistake that has now been corrected to 5%

5. Section 2.2. Were foraminifera from set 1 and set 2 combined for analysis, where they were grown under the same conditions?

We have now specified this in the manuscript by adding the sentence: “Individuals from set 1 and set 2, grown under the same conditions (same medium $[\text{SO}_4^{2-}]$), were not combined for analysis. They were measured separately”.

6. Section 2.3. I think it's fine to do so briefly, but please give some basic details of how the instruments were set up, exactly how the analyses were run, how the data were processed, what the blank data looked like etc. etc., rather than simply referring to a previous publication.

We have now added more details on geochemical analysis methods

7. Line 222. I'm not sure that this range (= ~50% of the modern ocean [DIC]) could really be considered fairly stable. I was also missing an explanation of why DIC was much higher in these experiments than natural seawater.

We removed the "fairly stable" mention and only refer to the range. We added the following explanation for the high DIC concentration:

"These concentrations are higher than the theoretical initial concentration of 2.8 mM using the recipe of Kester et al. 1967. While in Kester et al.'s recipe, the targeted 8.2 pH is achieved after 2h equilibration with the CO₂ in the atmosphere, we had to proceed to NaOH addition despite a 12h equilibration time. It is possible that higher CO₂ dissolution at the atmospheric pressure of the year we performed the experiments (407 ppm against 322 ppm in 1967), led to an increase in DIC. In addition, DIC probably built up in the Petri dishes each week as the foraminifera consumed the algae."

8. Section 3.3/figures. It might be helpful to report molar S/Ca ratios, to maintain consistency with the vast majority of the geochemical literature and previous work on foraminifera S.

We followed this suggestion and we reported in the figure 7 and in the appendix the values as molar S/Ca ratios

9. Line 248 "A $\delta^{34}\text{SCAS} - \delta^{34}\text{Ssw}$ fractionation value of $1.6 \pm 0.3\%$ was observed for For1C1 pool (9 samples in total coming from all [SO₄²⁻] concentrations) while it was $1.4 \pm 0.2\%$ for C1Tg specimens (9 samples in total coming from NSW or ASW[28]), which is indistinguishable within the error range (Fig. 7)". Please state what the uncertainty represents.

Thank you for pointing this out. The uncertainty is the 1sd calculated from each individual fractionation. We specify it in the text.

10. Lines 274-275. On the other hand, over the range that [SO₄²⁻] is thought to have varied within the Phanerozoic (~5-30 mM) there is no relationship between reproduction (growth?) and [SO₄²⁻].

In the range of Phanerozoic [SO₄²⁻] variations (~5-30 mM), the reproduction and growth of populations seems to be rather optimal. We added the sentence: "However, this appears to be for seawater [SO₄²⁻] variations far below and above the range (~5-30 mM) thought to be involved in long-term secular variations in the Phanerozoic, suggesting an adaptation of foraminifera in this range of variations. Indeed, under conditions that mimic the Phanerozoic range of [SO₄²⁻] variations, reproduction and population growth appear rather to be optimal."

11. Lines 286-288. Were the foraminifera crushed prior to cleaning? If not (possibly even if so) then inter/intra-crystalline organics likely remained.

Foraminifera were not crushed. We agree with the reviewer that organics likely remained, as stated in the text: "we assume that most of the measured [SO₄²⁻] in the tests are linked to the CAS concentration, although a small contribution might be still associated with S_{org} within the biomineralized calcite". We also performed analysis of foraminifera with organic carbon, which permit to tell that traces of organic carbon could have lowered the overall $\delta^{34}\text{S}$ value. But there is no reason to believe that different amount of inter/intra crystalline organics would have been preserved from one concentration to the other. This study main conclusion is thus focusing on the maintained fractionation factor between foraminiferal $\delta^{34}\text{S}$ to water sulfate $\delta^{34}\text{S}$ at each concentration, rather than discussing the absolute isotopic fractionation value.

12. Lines 294-296, lines 326-327 I found the comparison a little simplistic as of course pH/DIC/[Mg²⁺]/organics are also very important.

We tried to develop the discussion here (in blue) and added a few references:

“The putative mechanisms from i to iii for sulfate regulation could have been adopted by foraminifera as evolutionary strategies to maintain carbonate precipitation despite potential variation in [SO₄²⁻]. Indeed, at [SO₄²⁻] greater than 8 mM abiotic calcite nucleation and precipitation is inhibited, and aragonite precipitates from saturated solutions (Kitano and Hood, 1962; Kitano et al., 1975; Bots et al., 2011). This inhibition is also true in the lack in magnesium (Barkan et al., 2020) and thus sulfate alone can affect calcite precipitation. Mechanisms such as increasing calcium concentration, pH and/or saturation state (e.g. Zeebe and Sanyal, 2002; Nehrke et al., 2013; Evans et al., 2018), as well as the presence of organics, could help overcome such high concentration of sulfate. However, when it comes to magnesium, active removal is also an option (Bentov and Erez, 2006).”

13. Line 297, lines 322-324. I would also add a possible kinetic effect to the list. If crystal growth rates are lower at higher [SO₄²⁻], as the inorganic work indicates, all else being equal, then a nonlinear seawater-shell relationship might be expected.

We have now added a discussion of "kinetic effect" to the list, as follows: “A kinetic effect could also explain the non-linearity of the CAS concentration in foraminiferal tests with corresponding increases in [SO₄²⁻] above 28 Mm, as inorganic calcite precipitation experiments suggest a reduction in crystal growth rates at higher [SO₄²⁻]. However, it is worth noting that a decrease in precipitation rate can also be associated to a lower CAS content in inorganic calcite (Barkan et al., 2020). As a result, one could imagine that the change in sulfate concentration reflects a change in precipitation rate induced by different sulfate concentration in seawater and/or in the biomineralizing fluid. However, as calcite is more soluble and precipitates less easily at high sulfate concentration, we would expect an effect opposite to what we observe in the 5-40 mM part of our results. There could nonetheless be a contribution of the rate effect to the plateau we observe”

14. Line 315. Please rephrase. Kadan *et al.* studied coccolithophores, which have a completely different biomineralisation pathway (e.g. centred on transmembrane ion transport rather than seawater vacuolisation).

We have removed this part and this reference, and this part remains more focused on the formation of complexes in the precipitation liquid.

15. Line 381. Does it become lethal? Or does it simply prevent calcification?

We deleted lethal according also the comments of Julien Richirt.

16. Figure 6. Seawater SO₄²⁻/Ca²⁺ is unitless, and the /CaCO₃ is unnecessary on the y axis.

Thank you. We corrected the figure, though we preferred to keep the /CaCO₃ to avoid ambiguity.

Typos

1. Line 19. Calcifiers.

Corrected

2. Line 321. van Dijk.

Corrected

3. Line 578. de Nooijer.

Corrected