

egosphere-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.

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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.

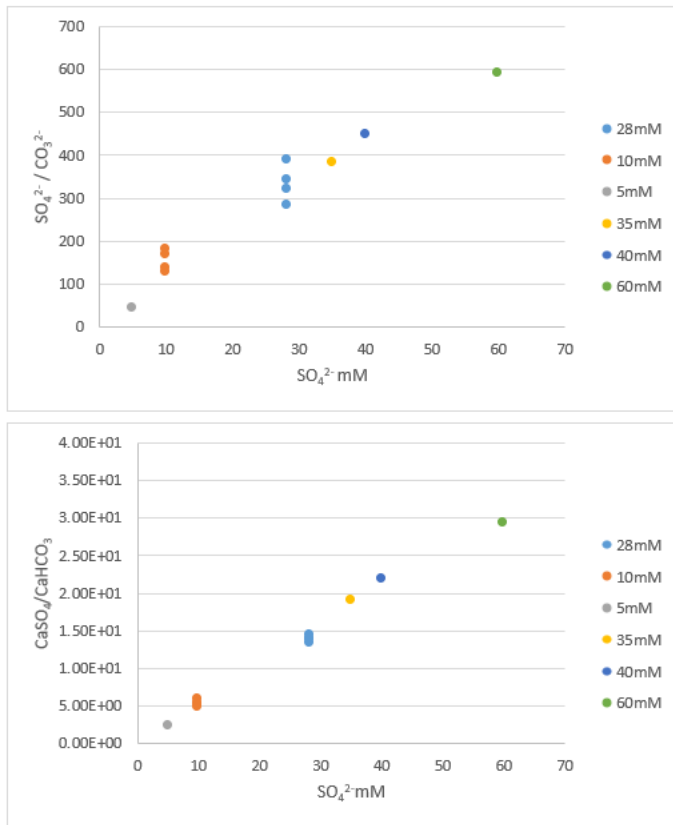
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The output data considered are Ca²⁺, all free and complexed SO₄²⁻ species, free DIC species and CaHCO₃. The sole DIC species present as a complex that was extracted is CaHCO₃, because it is the major complex. It is also a species that has been hypothesized to be potentially incorporated into calcite, as CaSO₄ could be.

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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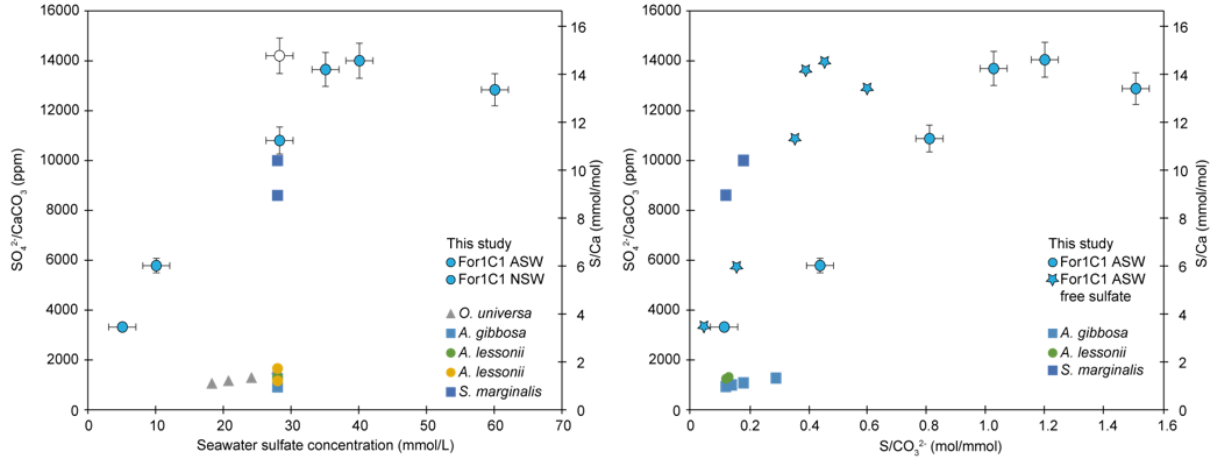


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Figure D1. Geochemical modeling results showing $\text{SO}_4^{2-} / \text{CO}_3^{2-}$ ratio and $\text{CaSO}_4 / \text{CaHCO}_3$ ratio in solution as a function of total sulfate concentration in solution. Each point corresponds to a different computational 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 S/CO₃²⁻. 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, S/CO₃²⁻ 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 $\delta^{34}\text{S}$ (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 $\delta^{34}\text{SCAS} - \delta^{34}\text{S}_{\text{sw}}$ fractionation value of $1.6 \pm 0.3\text{‰}$ was observed for For1C1 pool (9 samples in total coming from all [SO₄²⁻] concentrations) while it was $1.4 \pm 0.2\text{‰}$ 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 $\delta^{4}S$ to water sulfate $\delta^{4}S$ 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