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Impact of seawater sulfate concentration on sulfur concentration and isotopic composition in calcite of two cultured benthic foraminifera

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Abstract. Marine sediments can be used to reconstruct the evolution of seawater [SO_4^{2-}] and $\delta^{34}S$ over time, two key parameters that contribute to refine our understanding of the sulfur cycle and thus of Earth's redox state. $\delta^{34}S$ evolution can be measured from carbonates, barites and sulfate evaporites. [SO_4^{2-}] variations can be reconstructed using fluid inclusions in halites, a method that only allows a low-resolution record. Reconstruction of the past sulfur cycle could be improved if carbonates allowed to track both seawater $\delta^{34}S$ and [SO_4^{2-}] variations in a sole, continuous sedimentary repository. However, most primary carbonates formed in the ocean are biogenic, and organisms tend to overprint the geochemical signatures of their carbonates through a combination of processes often collectively referred to as vital effects. Hence, calibrations are needed to allow seawater $\delta^{34}S$ and [SO_4^{2-}] reconstructions based on biogenic carbonates. Because foraminifera are important marine calcifyers, we opted to focus on calcite synthesized by individuals of two benthic strains cultured in laboratory under controlled conditions, with varying seawater [SO_4^{2-}] (ranging from 0 mM to 180 mM). Our experimental design allowed us to obtain foraminiferal asexual reproduction over several generations. We measured bulk carbonate associated sulfate (CAS) content and sulfur isotopic composition ($\delta^{34}S_{CAS}$) on samples of tens to hundreds of specimens for each culture medium, where [SO_4^{2-}] varied from 5 to 60 mM. Increasing or decreasing [SO_4^{2-}] with respect to modern-day seawater concentration (28 mM) impacted foraminiferal population size dynamics and the total amount of bioprecipitated carbonate. 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. Yet, despite the significant effect of [SO_4^{2-}] on foraminiferal physiology and on CAS incorporation, the isotopic fractionation between CAS and seawater remains stable through varying seawater [SO_4^{2-}]. Altogether, these results illustrate that CAS in biogenic calcite could constitute a good proxy for both seawater [SO_4^{2-}] and $\delta^{34}S$ and contributes to emphasize the role played by sulfate on foraminiferal biomineralization and biological activity.

1 Introduction

In the modern ocean, marine organisms control the precipitation of most calcium carbonates through the biomineralization of calcite or aragonite, the two main $CaCO_3$ polymorphs. Biogenic calcium carbonates from the sedimentary record have been used for decades to reconstruct past environmental conditions. At modern sulfate and magnesium concentrations in seawater (about 28 mM and 50 mM respectively), aragonite precipitates preferentially over calcite in abiotic conditions (Bots et al., 2011; Barkan et al., 2020; Goetschl et al., 2019). Seawater sulfate and magnesium concentrations varied over the last 550 Myr, ranging from ~5 mM to ~28 mM (Horita et al., 2002), and from ~44 to ~55 mM, respectively (Lowenstein et al., 2001; Brennan et al., 2004). Lower

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and higher seawater sulfate and magnesium concentrations have been shown to match calcitic and aragonitic oceans, where calcite or aragonite forming organisms were favored respectively (Lowenstein et al., 2003; Algeo et al., 2015; Lin et al., 2018, Goestchl et al., 2019). In the modern aragonitic ocean (Sandberg et al., 1983) as well as through parts of the geological past of Earth's history, the occurrence of calcitic organisms (e.g. foraminifera, coccolithophorids, some mollusks, bryozoans and coralline algae) could thus appear as a paradox. These calcitic organisms growing in aragonite oceans with high sulfate content must then have developed adaptive strategies and exerted a high degree of biological control in calcite bioprecipitation and sulfate incorporation, which need to be better understood.

Among the main calcite synthesizers, foraminifera are unicellular eukaryotes that build mainly calcitic (rare aragonitic species exist) shells named "tests", that accumulate on the ocean seafloor (Schiebel 2002; Langer 2008). As foraminifera build their tests, trace elements present in seawater get incorporated in the biomineral structure. Sulfur is assumed to be incorporated in the calcium carbonate lattice structure as SO_4^{2-} by replacing a CO_3^{2-} group (Kontrec et al. 2004; Fernandez-Diaz et al. 2010) and is referred to as CAS for carbonate associated sulfate. This has been illustrated by an increase in S/Ca as a function of $[CO_3^{2-}]$ decrease in benthic foraminiferal calcite (van Dijk et al., 2017). Paris et al. (2014) evidenced that the planktic species *Orbulina universa* faithfully records the $[SO_4^{2-}]$ /$[Ca^{2+}]$ ratio of the seawater in which it grew for $[SO_4^{2-}]$ values from 18 mM to 28 mM. These encouraging results, however, needed to be tested on benthic species and on a wider range of $[SO_4^{2-}]$, to cover deep time oceanic values which varied from less than 5 mM to 28 mM nowadays (Algeo et al. 2015) and potentially beyond, during large volcanic events in the past, or in the vicinity of sulfate-rich volcanic hydrothermal fluids on the seabed (Gamo et al. 1997; Laakso et al. 2020). Furthermore, the possibility that foraminiferal calcite could serve both as $[SO_4^{2-}]$ and $\delta^{34}S$ record needs to be further validated. While so far measurements in biogenic carbonates showed that sulfur isotopes are systematically fractionated by ± 1 ‰ from seawater (Kampschulte et al., 2001; Paris et al., 2014; Present et al., 2015; Rennie et al., 2018), recent experiments of abiotic $CaCO_3$ precipitation showed that a 2-5 ‰ fractionation of sulfur isotopes between aqueous sulfate and CAS in calcite covary with $[SO_4^{2-}]$ and, to a lesser extent, with precipitation rates (Barkan et al., 2020). There is thus a contrasting abiotic-biotic behavior that needs to be elucidated in order to determine whether calcitic foraminiferal tests could be used as a paleoenvironmental archive for the sulfur cycle, and interrogate the possibility that seawater $[SO_4^{2-}]$ variations impact foraminiferal biocalcification and carbonate production.

To answer these questions, we grew two strains of Rosalinidae (Fig. 1), which are asymbiotic benthic foraminifera, at constant temperature, pH and salinity over a 180-fold range of seawater $[SO_4^{2-}]$. Compared to planktic foraminifera, benthic foraminifera have two advantages: (i) they cover deeper geological times and (ii) they can reproduce more easily in experimental conditions. In general, in both planktic and benthic foraminiferal culture experiments performed to calibrate geochemical proxies, populations of individuals captured in the wild do not have the time to adapt to the experimental conditions because maintaining foraminiferal reproductions over several generations is a complicated task. Therefore, measurements of geochemical proxies are usually performed either on the few test chambers that precipitated in the experimental medium (e.g. Dissard et al., 2010 a and b; van Dijk et al., 2017; Schmidt et al., 2022), or on whole tests that include the initial chambers grown in the natural marine environment prior to collection (e.g. Paris et al., 2014; Le Houedec et al., 2021). Our experiment was carefully designed to obtain several generations grown over several weeks in each experimental medium, ensuring both acclimatization and full precipitation of the test in the medium. We analyzed population size dynamics, as well as shell $\delta^{34}S$ and $[SO_4^{2-}]$ in bulk samples of tens to hundreds of specimens in each medium to shed light on the mechanisms of sulfate incorporation in benthic foraminiferal calcite.

2 Materials and methods

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2.1 Culture conditions

2.1.1 Long term culture with asexual reproduction

Culture experiments were conducted at the French National Museum of Natural History (Muséum national d'Histoire naturelle, MNHN) in the free living protist collection facilities (collection group: Biological Resources of Living and Cryopreserved Cells; Collection of Unicellular Eukaryotes) on two previously cloned foraminiferal strains adapted to *in vitro* cell culture in 90 mm Petri dishes with natural sea water (NSW) and fed with *Chlorogonium sp.* (specimen MNHN-CEU-2016-0001), a freshwater microalga. Two strains namely, For1C1 (specimen MNHN-CEU-2016-0075) and C1Tg (specimen MNHN-CEU-2016-0075) (Fig. 1), were isolated from the top layer of sediments collected from Banyuls sea shore (Mediterranean French coast) in 2006, and Concarneau (Atlantic French coast) in 2011, respectively.

Both strains were maintained through asexual reproduction (Fig. 2), using the following method: foraminifera were cultured in 90 mm Petri dishes filled with 0.22 µm filtered NSW from Banyuls, France for For1C1 strain, or Concarneau, France for C1Tg strains. The NSW was kept in a cold room for at least a month and its pH (NBS scale) was adjusted to 8.2 through addition of NaOH and/or HCl, before use. The Petri dishes were kept at 22°C in an incubator (Memmert IPP 110 plus) equipped with cold white light modules (5,500 K) with a 12h day-12h night cycle. Water in the Petri dishes was changed once a week and foraminifera fed with living freshwater algae (*Chlorogonium* sp.). The algae were cultured in Basal Bold medium at 25°C under medium light intensity, and suspended in sterile pH 8.2 NSW after 3 steps of rinsing with NSW. Using freshwater algae to feed foraminifera is an innovative approach particularly adapted for long term cultures and geochemical studies. Being 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 does not influence the seawater chemistry conditions within the Petri dishes. Every other week, a new Petri dish was set up with a dozen of new juveniles (pre-adults below the age of asexual reproduction, characterized by test with ~ 10 chambers). Live cultures were discarded after a month to prevent bacterial or fungal spread.

2.1.2 Culture in artificial seawater with varying [SO_4^{2-}]

In 2016, the two foraminiferal strains (For1C1 and C1Tg) were transferred to 0.22 µm filtered artificial seawater (ASW) mimicking NSW. The ASW was prepared following Kester et al. 1967. The total salinity was 35.06 g/L and the main ionic concentrations were as follow, in mM: Cl^- 543.9, Na^+ 467.3, SO_4^{2-} 28.2, Mg^{2+} 53.1, Ca^{2+} 9.9, K^+ 10.0, HCO_3^- 2.3, Br^- 0.8, H_3BO_3 0.4, Sr^{2+} 0.1, F^- 0.1. After equilibration with the atmosphere for 2 to 3 hours, the pH of ASW was adjusted to pH 8.2 by the addition of NaOH and HCl. ASW and NSW were sterilized by filtration on a 0.22 µm filter. The acclimation to ASW lasted approximately for a year (with foraminifera being transferred to new 90 mm Petri dishes monthly) without any noticeable effect on the foraminiferal life cycle and morphology. Over this period of time, batches of several hundreds of foraminifera of each species (strains For1C1 and C1Tg), cultured either in ASW or NSW, were sampled for [SO_4^{2-}] and $\delta^{34}S$ composition measurements.

To produce media with different [SO_4^{2-}], we created an ASW without SO_4^{2-} (hereafter ASW[0]) and another with [SO_4^{2-}] = 180 mM (hereafter ASW[180]). The amount of NaCl in those two media was adjusted to keep the total salinity constant (35.06 g/L). Na^+ concentrations for ASW[0] and ASW[180] were 479 mM and 402 mM, respectively and while the Cl^- concentrations were 612 mM and 175 mM, respectively. ASW[0] and ASW[180] were mixed in various proportions in order to obtain 8 other ASW with the following [SO_4^{2-}]: 1, 5, 10, 35, 40, 50, 60, 90 and 120 mM. Each of these media had the same salinity as ASW (35.06

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g/L), pH (8.2), DIC (Dissolved Inorganic Carbon: $[CO_2]+ [H_2CO_3]+[HCO_3^-]+[CO_3^{2-}]$) and ALK (Alkalinity). For1C1 was the sole strain grown under different SO_4^{2-} concentrations (Table 1).

In a first set of experiments (Set 1), 17 to 31 For1C1 individuals (Table 1) with ~ 10 chambers each were transferred from ASW to new 60 mm Petri dishes filled with the following media: ASW (hereafter ASW[28]), ASW[0], ASW[5], ASW[10], ASW[60], ASW[120] and ASW[180] and then cultured for 34 days. In parallel, 17 individuals so far cultured in natural seawater were moved to a new 60 mm Petri dish containing NSW from Banyuls and were cultured for 39 days.

In a second set of experiment designed to extend our concentrations range (Set 2), 6 individuals of For1C1, also presenting ~10 chambers each, were transferred from ASW to new 60 mm Petri dishes and were cultured for 33 days in the following media ASW[28], ASW[1], ASW[10], ASW[35], ASW[40], ASW[50], and ASW[90] (Table 1).

For large populations, the specimens were distributed over several 60 mm Petri dishes (up to 3) to avoid problems associated with superpopulation. In both sets of experiment, chlorogonium fed to foraminifera were rinsed and suspended in the media corresponding to each petri dishes prior to their addition. We counted live individuals each week, for each medium. Since the studied species live attached to a substrate, individuals that no longer stick to the Petri dishes were considered dead, even though some dead individuals (empty tests or no reticulopodial activity) may remained attached and few living adults can detach themselves from the substrate as well. After counting and discarding the dead individuals, we sampled 6 mL of water through a 0.2 µm filtered for DIC and $[SO_4^{2-}]$ measurements in gastight Exetainer© tubes full to the brim and stored at 5 °C. Consecutively, pH was measured using a Hach PHC281101 probe calibrated following the three points procedure (Hach singlet solutions calibrated against NIST standards, precision of ± 0.01 pH unit). Finally, the old water was completely replaced by fresh sterile water.

2.1.3 DIC analyses

DIC analyses were performed using 3 mL samples of seawater that were slowly withdrawn from each assay through the Exetainer© rubber septa using needles syringes. Ultra-pure helium gas was injected in each vial during sampling to ease solution withdrawal and to prevent atmospheric CO_2 contamination. Each 3 mL sample was injected into a new Exetainer© vial, previously flushed with ultra-pure helium gas (2.5 bar) and loaded with 0.3 mL of 100% H_3PO_4. Acidification with pure H_3PO_4 converts the total DIC of the sample into gaseous CO_2 which was allowed to degas and mix with the helium gas overnight under shaking. The CO_2 and He mix was then sampled with an autosampler and sent to a Dual Inlet FinniganTM DeltaPlus XP isotope ratio mass spectrometer (Thermo Fisher Scientific) (reproducibility = ±0.05‰) at IPGP, Paris. [DIC] was quantified using the linear relationship between DIC concentration and intensity of the m/z 44 ($^{12}C^{16}O^{16}O$) peak provided by the mass spectrometer (Assayag et al., 2006). This linear relationship was established based on repeated analyses of internal laboratory carbonate standards calibrated against international standards (100% calcite), run in different aliquots. The reproducibility for [DIC] measurements was ± 5% of the measured values (1σ).

2.2 Collection and rinsing procedure of the tests for geochemical analyses

At the end of the culture experiments (that varied between 34 and 39 days), all individuals of the strain For1C1 from each Petri dish were recovered. Each sample typically weighed few milli-grams. The collected tests were rinsed 3 times in MQ water (basified to pH 9.5 with NH_4OH) to remove all traces of salts without dissolving the carbonate phase. In order to remove fresh organic matter, foraminifera were cleaned following Paris et al. (2014): foraminifera were bathed in a NaOH (0.5M) + H_2O_2 (15%) solution

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at 60°C for 30 minutes. They were then rinsed three times in basified MQ water, and dried overnight at 50°C in a drying oven. All samples were then dissolved at CRPG (Nancy) in 0.5 ml of 1 to 2% HCl. In addition, in order to determine how the remaining traces of organic matter could affect $\delta^{34}S$ measurements, some individuals from both For1C1 and C1Tg strains were dissolved in Aqua Regia (a 50/50 mix of concentrated HNO_3 and HCl), without prior cleaning in NaOH and H_2O_2. They were left overnight at 120°C and dried down. All acids were distilled at CRPG and the 18.2 MΩ water purified through a Helga device (Veolia).

2.3 Geochemical analyses

2.3.1 CAS concentration analysis

Two dissolved foraminiferal calcite aliquots of 50 µl were used to determine sulfate and calcium concentrations of the samples. To measure [SO_4^{2-}], one of the 50 µl aliquot was diluted in 200 µl of 18.2 MΩ water and ran on a Metrohm ion chromatography system (ICS). The calcium content of the samples was measured using a X-series II ICP-MS using the second aliquot that was dried down and taken up in 3 ml of 2% HNO_3.

2.3.2 $\delta^{34}S$ analysis

Sulphate isolation from the carbonate matrix was performed by ionic chromatography using the sulfate specific resin anionic resin Biorad AG1X8 (Paris et al., 2014) using precleaned disposable Biorad columns. Each column was prepared by loading 0.6 ml of resin and rinsed with 10% V/V HNO_3 (2x10CV - 1 CV = 1 column volume = 0.6 ml), 33% V/V HCL (2x10CV), 0.5N HCl (1x10CV). After introducing the dissolved carbonates sample on the resin, the column was rinsed with ultrapure water (5x5CV) to remove cations. Sulphate was then eluted with 0.45 M HNO_3 (3x2CV). Each batch of columns included a sample of 50 µl of seawater as a reference and total procedure blanks. After elution, samples were dried down on hotplates with open lids (105°C). Purified samples were analysed on the ThermoScientific Neptune Plus MC-ICP-MS at the CRPG using a standard-sample bracketing method (Paris et al, 2013). Samples were run at high resolution using an Aridus-II desolvating membrane to decrease oxide and hydride interferences. Data were then corrected for instrumental fractionation, drift and background following Paris et al. 2013. The bracketing Na_2SO_4 solution had been previously calibrated against international standard IAEA S1 and checked against IAEA S2 and S3. Seawater samples ran during each Neptune sessions ensure accuracy of the data. The reproducibility for $\delta^{34}S$ measurements was ± 0.2‰ of the measured values (2σ).

3 Results

3.1 Population growth in each medium

Individuals morphologically similar to *Rosalina* (Fig. 1) reproduced asexually when their tests reached a development of 11-12 chambers (Fig. 2 and Fig. 3). Under standard culture conditions with low cell density, the reproductive cycle lasted ~ 12 to 15 days and individuals "died" after asexual reproduction by dividing themselves, usually into 20 to 40 viable juveniles, leaving an empty test (Fig. 2). Adult specimens were smaller than the traditional foraminiferal fraction obtained from sieving (through >125 µm mesh) in geochemical studies, they thus may well be common and rarely collected because of their size. A morphological and

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taxonomic description of the cultured strains is available in the Appendix A. The weekly number of accumulated live individuals incremented by reproduction is given in Fig. 4 and in the Appendix B Table B1.

The number of accumulated individuals can trace the population size dynamics for each medium and depends on reproduction rate, number of individuals produced by the same cell and mortality. However, while the increase in the number of individuals clearly shows that living cells are being produced, no certainty about their viability can be drawn when the number of individuals stagnates or decreases, as no vital staining has been performed. It was therefore not always easy to distinguish between inactive and dead cells. We inferred mortality from the cessation of reticulopodial activity and cytoplasmic streaming, as well as from the change in cell color (Bernhard, 2000). In the media with no sulfate or sulfate concentrations above 60 mM, we observed little to no reproduction, cell inactivity and probably mortality. As a result, the number of attached foraminifera remained constant and/or decreased over time (Fig. 4, Appendix B Table B1). The most dramatic reactions were observed within a few hours in the media with highest [SO_4^{2-}] (ASW[120] and ASW[180]), where individuals did not reproduce nor even show any reticulopodial activity. In ASW[90] and ASW[1], only one reproduction cycle was observed and after few days all the cells were inactive (Fig.4, Appendix B Table B1). Overall, the highest numbers of individuals at the end of the experiment were obtained in the ASW[28], NSW (Banyuls), ASW[10] and ASW[35] media (Fig. 4). We also note that the number of foraminifera in ASW[10] was higher in Set 2 than it was in Set 1 even though the medias were identical. The only difference was the initial number of foraminifera for each experiment, with 6 individuals in Set 2 instead of 28 as in Set 1. It is likely that each of the 6 individuals was chosen more carefully, which could induce a bias and may explain a more active behavior of the foraminifera compared to Set 2. Even under this condition, the bias would nonetheless be systematic and similar for each medium (all Petri dishes in set 2 started with 6 foraminifera) and thus do not prevent comparing the results within the set.

3.2 pH and DIC evolution

pH variations remained within ± 0.2 pH units during each experiment (Table 2).

pH drifted from the starting point between 8.1 and 8.2 towards more acidic values (7.83 minimum) and was reset close to 8.2 at each medium change for the first 15 days and then remained rather stable with values varying between 8.19 and 8.07. DIC remained fairly stable in each medium with values ranging from 3.2±0.2 mM (2σ) to 4.2±0.3 mM (2σ) (Table 3). pH and DIC variations for cultures in ASW[28] and ASW[10] are shown in Fig. 5.

3.3 CAS concentration

CAS concentration in foraminiferal calcite for each medium when enough tests could be collected for analyses (i.e. ASW[5], ASW[10], ASW[28], ASW[35], ASW[40] and ASW[60]) is presented in Fig. 6 and Table 4. Each datapoint was obtained using hundred to several hundreds of foraminifera for each medium. CAS concentration (sulfate to calcite ratio) increased from 3320 ppm to 14000 ppm $SO_4^{-2}/CaCO_3$ (±5%, 2σ) in proportion to SO_4^{2-} concentrations in artificial seawater which increased from 5 mM to 40 mM. While at 60 mM of [SO_4^{2-}] in seawater CAS content in foraminifera decreased to 9740 ppm, indicating that a threshold value of CAS of 14000 ppm was probably reached at 40 mM of [SO_4^{2-}] in seawater. CAS concentration of foraminifera grown in artificial or natural seawater under the same SO_4^{2-} concentration are considered identical within error (Fig. 6).

3.4 Sulfur isotopic composition

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The $\delta^{34}S$ values of the foraminiferal CAS from the different media are plotted in Fig. 7 and listed in Table 4. Measurements were performed both in foraminifera specimens cultured in NSW or ASW[28] during the acclimation period and in those coming from the $[SO_4^{2-}]$ variation experiment, when enough specimen could be collected (Table 4). 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. Considering that algae $\delta^{34}S$ composition are of 7.0±0.2‰ the difference may be explained by the isotopically depleted sulfate added resulting of algae decomposition, lowering the average $\delta^{34}S$ of the media. This effect was not detectable in ASW possibly because the $\delta^{34}S$ values of medium (9.1±0.2‰ for ASW[28] and 0.1±0.2‰ for ASW[5], ASW[10], ASW[35], ASW[40] and ASW[60]) was closer to that of the algae (Appendix B Table B2). Considering that algae were added at each water change and degraded within 1 or 2 days, and that foraminifera entered into a chamber formation sequence after feeding (Fig. 3), we consider that the seawater $\delta^{34}S$ that prevailed during chamber formation is the value measured after several days of culture with algae, 19.9±0.2‰ in NSW and 9.1±0.2‰ or 0.1±0.2‰ in ASW[28] (Appendix B Table B2). A $\delta^{34}S_{CAS}$ - $\delta^{34}S_{sw}$ fractionation value of 1.6 ±0.3‰ was observed for For1C1 pool (9 samples in total coming from all $[SO_4^{2-}]$ concentrations) while it was 1.4±0.2‰ for C1Tg specimens (9 samples in total coming from NSW or ASW[28]), which is indistinguishable within the error range (Fig. 7).
Samples for which organic matter was preserved yielded $\delta^{34}S$ values of 1.1±0.2‰ (For1C1 in ASW[28]) 0.4±0.2‰ (For1C1 in NSW) 1.4±0.2 ‰ (C1Tg in NSW) and 0.5±0.2‰ (C1Tg in ASW[28]) lower than the value that was obtained for the For1C1 Banyuls and C1Tg Concarneau tests from which organic matter had been oxidatively removed (Table 4 and Fig. 7).

4 Discussion

4.1 $[SO_4^{2-}]$ changes in seawater can affect foraminiferal biology

Our results highlight that a change in seawater $[SO_4^{2-}]$ concentration can affect foraminiferal biology and population size dynamics. Reticulopodial activity stopped few hours after the transfer of the foraminifera from 28 mM of sulfate (ASW[28]) to concentrations above 120 mM (ASW[120] and ASW[180]) or without sulfate (ASW[0]). Dissolved sulfate and food were the only sources of sulfur in this experiment. Since ASW[0] prevented any reproduction and induced cellular inactivity, an important inference is that sulfur from food appears insufficient and that dissolved sulfate in seawater is necessary for cellular activity in foraminifera. At the other extreme, toxic impact of the highest $[SO_4^{2-}]$ (ASW[120] and ASW[180]) can explain the non-reproduction and the cellular inactivity of individuals after a few hours. Foraminifera survived and even reproduced once in the ASW[1] and ASW[90] media (Fig. 4). Thus, our results suggest that foraminifera can sustain their physiological activity only within a certain range of $[SO_4^{2-}]$, from 1 to 90 mM, extreme values at which the activity is already very low.
In this experiment, the highest number of weekly accumulated live individuals was observed at the modern oceanic $[SO_4^{2-}]$ (28.2 mM) in both artificial and natural seawater, suggesting that these species are highly adapted to their actual environment. Population size decreased above and below that concentration, suggesting a foraminiferal physiological sensitivity to $[SO_4^{2-}]$ variation.
The effect of changes in seawater $[SO_4^{2-}]$ on physiology highlights a possible mechanism by which changes in the composition of seawater can affect the carbonate record. It has previously been hypothesized that seawater Mg/Ca ratio and SO_4^{2-}/Ca ratio, control the switch between calcite and aragonite dominance in the sedimentary record, as a high SO_4^{2-} and Mg concentrations in seawater inhibit calcite precipitation and promote aragonite precipitation, as shown by inorganic precipitation experiments (Bots et al., 2011; Barkan et al., 2020). Here, we show that a change in seawater $[SO_4^{2-}]$ may also affect foraminiferal physiology and therewith foraminiferal population size and their calcite accumulation in the sediment.

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4.2 Foraminifer CAS concentration versus seawater [SO_4^{2-}]

Sulfur in foraminiferal calcite have two possible locations:

i) CAS: Sulfate is incorporated into both inorganic and biogenic $CaCO_3$ minerals as CAS within the growing mineral structure, the larger tetrahedral sulfate substituting to the smaller trigonal-planar carbonate ion (Busenberg and Plummer, 1985; Kontrec et al. 2004; Balan et al., 2014; Tamenori et al. 2014; Perrin et al. 2017; Tamenori and Yoshimura 2018).

ii)S_{org}: Sulfur present in the organic matrix used by biomineralizing organisms to initiate calcification and orient the growing crystals (e.g. Cuif et al., 2004; Richardson et al., 2019; de Noojier et al., 2014). The organic matrix contains over-sulfated glycosaminoglycans and proteins (Weiner and Erez,1984; Langer 1992). In the case of rotaliid test, the calcareous wall growth of each new chamber results from the bioprecipitation of two calcite layers, on either side of an organic matrix (Bé et al., 1979; de Noojier et al., 2014; Nagai et al., 2018) referred to as the Primary Organic Sheet (POS, Erez, 2003). However, since we applied an oxidative cleaning to the foraminiferal tests to destroy the organic matter, we assume that most of the measured [SO_4^{2-}] in the tests are linked to the CAS concentration, although a small contribution might be still associated with S_{org} within the biomineralized calcite (Burdett et al., 1989; Cuif et al., 2003; Paris et al. 2014). In the following discussion, we will thus assume that our measured sulfate content reflects structurally-bound CAS.

Our results show that CAS concentration in foraminiferal calcite linearly increases with seawater [SO_4^{2-}] concentration from 5 to 28 mM (Fig. 6), similarly to what is observed in inorganic carbonates (Busenberg and Plummer, 1985; Fernandez-Diaz et al., 2010; Barkan et al., 2020) or previous foraminiferal investigation (Paris et al., 2014). At [SO_4^{2-}] higher than 28 mM in sea water, the incorporation of sulfate in the foraminifer calcite seems to reach a saturation point (Fig. 6). It is remarkable to note that foraminifera can reproduce and thus calcify at [SO_4^{2-}] as high as 90 mM (Fig. 4), concentrations at which no inorganic calcite precipitation occurs (Bots et al., 2011; Barkan et al., 2020).

Several hypotheses can be formulated to explain this $SO_4^{-2}/CaCO_3$ incorporation pattern:

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 the concentration, making calcite nucleation possible. In fact, under our experimental conditions the amount of CAS incorporated in foraminiferal calcite is correlated to increase in seawater SO_4^{2-} concentration (up to 28 mM) and probably indicates that sulfate is only partially removed from the precipitating fluid, altering the local SO_4^{2-} concentration that is proportional to that of seawater SO_4^{2-}.

ii) An increase in the carbonate ion concentration may help maintaining a constant SO_4^{2-}/CO_3^{2-}. Previous investigations demonstrated that it is more appropriated to reason in terms of SO_4^{2-}/CO_3^{2-} ratio of the calcifying fluid rather than [SO_4^{2-}] as sulfate substitutes for CO_3^{2-} in the forming mineral (van Dijke et al., 2019; Barkan et al., 2020). Another way to maintain SO_4^{2-}/CO_3^{2-} constant while [SO_4^{2-}] increases would be to proportionally increase CO_3^{2-}. Like other calcifying organisms, benthic foraminifera modify the pH of the precipitating fluid to promote calcite formation (Erez, 2003; de Nooijer et al., 2008; Rollion-Bard and Erez, 2010; Toyofuku et al., 2017). Foraminifera, most probably actively pump protons out of the SOC (Sabbatini et al., 2014; Toyofuku et al., 2017). Intensifying this process in case of elevated [SO_4^{2-}] would induce an increase in carbonate ion concentration (and the saturation state) and therefore could help maintaining a constant SO_4^{2-}/CO_3^{2-} when [SO_4^{2-}] reaches 40 mM.

iii) Another possibility to explain why the CAS in foraminiferal tests does not increase linearly with corresponding increase in the [SO_4^{2-}] beyond 28 Mm, could be that at such concentrations in solution, sulfate might complex more easily with other cations such as Ca^{2+}, K^+, Mg^{2+}, Na^+, Sr^{2+}, etc. (Garrels and Thompson, 1962). Such complexes cannot be effectively incorporated into the mineral lattice structure. This might influence the amount of SO_4^{2-} substituted in carbonates, and thus the CAS in foraminiferal

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tests. Considering that the latest publications tend to point out a calcifying fluid made of a pseudogel filled with organics (Kadan et al., 2020), polymerization/depolymerization of organics controlled by the organism could as well affect SO_4^{2-} osmolarity and thus its propensity to form complexes.

iv) Finally, a preferential sequestering of sulfate in some organic rich layers at the incipient phase of biocalcification might allow to decrease the [SO_4^{2-}] in the remaining liquid and thereby prevent sulfate incorporation over 40 mM. High resolution sulfur nano-mapping on transversal section of perforate foraminiferal tests (such as Rosalinidae or *Orbulina*) showed a banded heterogeneity in sulfur distribution across the multi-layer structure (Paris et al., 2014; van Dijke et al., 2019). XRF intra-test mapping revealed a preferential incorporation of metals and sulfur in the POS zone, the organic incipient stage of the build-up of the wall of a new chamber of test (Lemelle et al., 2020). In our case, organic matter has been oxidized, and most of the "stored" SO_4^{2-} was likely removed.

All of these putative mechanisms for sulfate regulation could have been adopted by foraminifera as evolutionary strategies to maintain carbonate precipitation despite potential variation in [SO_4^{2-}]. Indeed, at [SO_4^{2-}] 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). Calcitic foraminifera, which first appeared during the Devonian (Vachard et al., 2010) in a range of low-sulfate seawaters of ~3-15 mM (Algeo et al., 2015), might have progressively adopted such strategies in order to precipitate calcite in high-sulfate (~28 mM) seawaters such as those in the present-day ocean, and retain the capacity to precipitate calcite at concentrations reaching 90mM as evidenced here.

4.3 Sulfur isotope fractionation

The isotopic composition of CAS remains constants through our experiments. Sulfur isotopic fractionation of CAS in benthic foraminifera (Rosalinidae) is not sensitive to the variation in [SO_4^{2-}] in seawater (Fig. 7), thus confirming the earlier observation on planktic foraminifera by Paris et al., 2014. This result by itself is important and confirms that foraminiferal CAS constitutes a reliable proxy of seawater $\delta^{34}S$. This result, together with the correlation between $SO_4/CaCO_3$ and seawater [SO_4^{2-}] (Fig. 6), supports that CAS in foraminiferal tests is of inorganic origin.

More importantly, the fractionation observed here is significantly different from the inorganic fractionation measured in the inorganic calcite (Barkan et al., 2020) highlighting the involvement of some biological isotopic fractionation. Considering that the organic sulfur source had a fixed sulfur composition (6.9‰) while the seawater $\delta^{34}S$ varied from one medium to the other (from -0.1 to 20.0‰), our isotopic measurements on S_{org}+CAS allow to infer the origin of S_{org} as well. Mass balance calculation permit to determine that the isotopic composition of S_{org} varies with seawater $\delta^{34}S$ value, pointing towards mainly an inorganic source for S_{org} (Fig.7). This is consistent with our observation that no cellular activity of foraminifera was possible in medium with zero [SO_4^{2-}], even in the presence of algae as food and possible source of S_{org}. The $\delta^{34}S$ value of the combined S pool (S_{org} + CAS) is 0.4 to 1.4 ‰ more negative than the $\delta^{34}S$ value of CAS alone, which points towards the involvement of some biological fractionation or vital effect associated to the incorporation of sulfur.

4.4 Implication for Paleoenvironmental reconstructions

Sulfur isotopic composition in the sedimentary record, through sulfur species redox reactivity and multiple deposition form, records several paleoenvironmental processes occurring in the atmosphere and the ocean (Farquhar et al., 2000; Farquhar and Wing, 2003;

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Crockford et al., 2019). This makes sulfur one of the most studied elements for the surface processes. And yet, in order to investigate the sulfur cycle, it is necessary to interrogate different sedimentary archives: carbonates, barites, evaporites as well as pyrites (Paytan et al., 1998; Algeo et al., 2015; Halevy et al., 2012; Present et al., 2020) to reconstruct variations in both $\delta^{34}S$ and $[SO_4^{2-}]$. The work to match the sedimentary record of both $\delta^{34}S$ and $[SO_4^{2-}]$ is laborious and requires calibrations. Our results indicate that foraminiferal calcite incorporate CAS proportionally to the $[SO_4^{2-}]$ in seawater, indicating that foraminiferal calcite could serve as a proxy for variations of both $\delta^{34}S_{CAS}$ and $[SO_4^{2-}]$ in seawater, particularly considering that the threshold value of 40 mM where CAS concentration reaches a plateau is much higher than any $[SO_4^{2-}]$ documented through Earth's history. The use of CAS concentration as a marine $[SO_4^{2-}]$ record is thus promising but will require calibration on various types of carbonates and species that may each have their own fractionation factor. The preservation of that dual $\delta^{34}S/[SO_4^{2-}]$ in foraminiferal calcite has to be evaluated in the carbonate record, as diagenesis has the capacity to affect $[SO_4^{2-}]$ in carbonates (e.g. Gill et al., 2008; Marenco et al., 2008; Rennie and Turchyn, 2014).

Additionally, it has been previously supported that S/Ca can work as a proxy for CO_3^{2-} concentration (van Dijke et al., 2017). Our results complement this finding under the condition that it is applied on timescales where seawater $[SO_4^{2-}]$ are constant.

The other major consequence of our results for the interpretation of the geological record is that changes in seawater $[SO_4^{2-}]$, can affect the production of carbonate by affecting the biology of certain organisms. In theory, the increase in seawater $[SO_4^{2-}]$ thermodynamically inhibits calcite formation. In this experiment we showed that higher $[SO_4^{2-}]$ in the media than those of the modern ocean can also decrease the amount of accumulated calcite by affecting foraminiferal population size. This work illustrated how the current and past crisis that affected biomineralizing organism communities by bringing conditions inhibiting carbonate formation in general, such as ocean acidification, or more specifically calcite formation, such as the increase in marine concentration of Mg^{2+} or SO_4^{2-}, can have both chemical "abiotic" effect on carbonates formation but also affect biological processes involved in biomineralization.

5 Conclusion

We cultured rotaliid foraminifera in media with $[SO_4^{2-}]$ spanning from 0 mM to 180 mM, stable salinity and fixed seawater $\delta^{34}S$. $[SO_4^{2-}]$ changes in seawater affected foraminiferal biology, population size dynamics and calcite accumulation. Foraminifera kept precipitating calcite in media reaching $[SO_4^{2-}] = 90$ mM. Sulfate from seawater is necessary for the cellular activity of foraminifera, but at concentrations above 90 mM it becomes toxic and lethal to them. Sulfur concentration in CAS varied proportionally to seawater $[SO_4^{2-}]$ between 1 and 28.2 mM and then stabilizes. Our results highlight that isotope fractionation between CAS and seawater does not depend on seawater $[SO_4^{2-}]$. Overall, similarly to planktic foraminifera the $\delta^{34}S_{CAS}$ value of a given species of benthic foraminifera is a reliable way to reconstruct seawater $\delta^{34}S$, despite variations of $[SO_4^{2-}]$ in seawater.

Author contribution: All authors participated in designing and interpreting the experiments. CT carried out culture experiments under MD and AB supervision and technical support from AL. MD isolated the strains and developed the cultivation protocol. GP performed all isotopic and $SO_4/CaCO_3$ analyses. CT designed the figures and wrote the paper with contributions from all co-authors.

Competing interests:

The authors declare no competing interests

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References

Algeo, T. J., Luo, G. M., Song, H. Y., Lyons, T. W., and Canfield, D. E.: Reconstruction of secular variation in seawater sulfate concentrations, Biogeosciences, 12, 2131-2151, https://doi.org/10.5194/bg-12-2131-2015, 2015.

Assayag, N., Rivé, K., Ader, M., Jézéquel, D., and Agrinier, P.: Improved method for isotopic and quantitative analysis of dissolved inorganic carbon in natural water samples, Rapid Communications in Mass Spectrometry, 20, 2243-2251, 2006.

Balan, E., Blanchard, M., Pinilla, C., and Lazzeri, M.: First-principles modeling of sulfate incorporation and $^{34}S/^{32}S$ isotopic fractionation in different calcium carbonates, Chemical Geology, 374-375, 84-91, https://doi.org/10.1016/j.chemgeo.2014.03.004, 2014.

Barkan, Y., Paris, G., Webb, S. M., Adkins, J. F., and Halevy, I.: Sulfur isotope fractionation between aqueous and carbonate-associated sulfate in abiotic calcite and aragonite, Geochimica et Cosmochimica Acta, 280, 317-339, https://doi.org/10.1016/j.gca.2020.03.022, 2020.

Bé, A. W., Hemleben, C., Anderson, O. R., and Spindler, M.: Chamber formation in planktonic foraminifera. Micropaleontology, 25, 294-307, https://doi.org/10.2307/1485304, 1979.

Bernhard, J. M.: Distinguishing live from dead foraminifera: Methods review and proper applications, Micropaleontology , 46, Suppl. 1, 38-46, 2000.

Bots, P., Benning, L., Rickaby, R., and Shaw, S.: The role of SO4 in the switch from calcite to aragonite seas, Geology, 39, 331-334, https://doi.org/10.1130/G31619.1, 2011.

Brennan, S. T., Lowenstein, T. K., and Horita, J.: Seawater chemistry and the advent of biocalcification, Geology 32, 473-476, https://doi.org/10.1130/G20251.1, 2004.

Burdett, J. W., Arthur, M. A., and Richardson, M.: A Neogene seawater sulfur isotope age curve from calcareous pelagic microfossils, Earth and Planetary Science Letters, 94, 189-198, https://doi.org/10.1016/0012-821X(89)90138-6, 1989.

Busenberg, E. and Plummer, L. N : Kinetic and thermodynamic factors controlling the distribution of SO_3^{2-} and Na^+ in calcites and selected aragonites, Geochimica et Cosmochimica Acta, 49, 713-725, https://doi.org/10.1016/0016-7037(85)90166-8, 1985.

Crockford, P. W., Kunzmann, M., Bekker, A., Hayles, J., Bao, H., Halverson, G. P, Peng, Y., Bui, T. H., Cox, G. M., Gibson, T. M., Wörndle, S., Rainbird, R., Lepland, A., Swanson-Hysell, N. L., Master, S., Sreenivas, B., Kuznetsov, A., Krupenik, V., and Wing, B. A.: Claypool continued: Extending the isotopic record of sedimentary sulfate. Chemical Geology, 513, 200-225, https://doi.org/10.1016/j.chemgeo.2019.02.030, 2019.

Cuif, J. P., Dauphin, Y., Doucet, J., Salome, M., and Susini, J.: XANES mapping of organic sulfate in three scleractinian coral skeletons, Geochimica et Cosmochimica Acta, 67, 75-83, https://doi.org/10.1016/S0016-7037(02)01041-4, 2003.

Cuif, J. P., Dauphin, Y., Berthet, P., and Jegoudez, J.: Associated water and organic compounds in coral skeletons: quantitative thermogravimetry coupled to infrared absorption spectrometry, Geochemistry, Geophysics, Geosystems, 5, Q11011, https://doi.org/10.1029/2004GC000783, 2004.

Dettmering, C., Rottqer, R., Hoheneqqer, J., and Schrnaljohann, R.: The Trimorphic Life Cycle in Foraminifera: Observations from Cultures allow New Evaluation, Europ. J. Protistol. 34, 363-368, 1998.

https://doi.org/10.5194/egusphere-2023-631
Preprint. Discussion started: 6 April 2023

Dissard, D., Nehrke, G., Reichart, G J., Bijma, J.: The impact of salinity on the Mg/Ca and Sr/Ca ratio in the benthic foraminifera *Ammonia tepida*: results from culture experiments, Geochimica et Cosmochimica Acta, 74, 3-74, doi:10.1016/j.gca.2009.10.040.

Dissard, D., Nehrke, G., Reichart, G.-J., Bijma, J.: Impact of seawater pCO(2) on calcification and Mg/Ca and Sr/Ca ratios in benthic foraminifera calcite: results from culturing experiments with *Ammonia tepida*, Biogeosciences, 7, 81-93, doi:10.5194/bg-7-81-2010.

Erez, J.: The source of ions for biomineralization in foraminifera and their implications for paleoceanographic proxies, in: Biomineralization, edited by: Dove, P. M., De Yoreo J. J., and Weiner, S., De Gruyter, Berlin, Boston, Reviews in Mineralogy & Geochemistry, 54, 115–149, https://doi.org/10.1515/9781501509346-010, 2003.

Farquhar J, Bao, H. M. and Thiemens, M.: Atmospheric influence of Earth's earliest sulfur cycle, Science 289, 756–759, DOI: 10.1126/science.289.5480.756, 2000.

Farquhar, J. and Wing, B. A.: Multiple sulfur isotopes and the evolution of the atmosphere, Earth and Planetary Science Letters, 213, 1-13, https://doi.org/10.1016/S0012-821X(03)00296-6, 2003.

Fernández-Díaz, L., Fernández-González, Á., and Prieto, M.: The role of sulfate groups in controlling $CaCO_3$ polymorphism, Geochimica et Cosmochimica Acta, 74, 6064-6076, https://doi.org/10.1016/j.gca.2010.08.010, 2010.

Gamo, T., Okamura, K., Charlou, J.-L., Urabe, T., Auzende, J.-M., Ishibashi, J., Shitashima, K., and Chiba, H. : Acidic and sulfate-rich hydrothermal fluids from the Manus back-arc basin, Papua New Guinea, Geology, 25, 139-142, 1997.

Garrels, R. M. and Thompson, M. E.: A chemical model for sea water at 25 degrees C and one atmosphere total pressure, American Journal of Science, 260, 57-66, https://doi.org/10.2475/ajs.260.1.57, 1962.

Gill, B. C., Lyons, T. W., and Frank, T. D.: Behavior of carbonate-associated sulfate during meteoric diagenesis and implications for the sulfur isotope paleoproxy, Geochimica et Cosmochimica Acta, 72, 4699-4711, https://doi.org/10.1016/j.gca.2008.07.001, 2008.

Goetschl, K. E., Purgstaller, B., Dietzel, M., and Mavromatis, V.: Effect of sulfate on magnesium incorporation in low-magnesium calcite, Geochimica et Cosmochimica Acta, 265, 505-519, https://doi.org/10.1016/j.gca.2019.07.024, 2019.

Halevy, I., Peters, S. E., and Fischer, W. W.: Sulfate burial constraints on the Phanerozoic sulfur cycle, Science, 337, 331-334, DOI: 10.1126/science.1220224, 2012.

Horita, J., Zimmermann, H., and Holland, H.D.: Chemical evolution of seawater during the Phanerozoic: Implications from the record of marine evaporites, Geochimica et Cosmochimica Acta 66, 3733–3756, 2002.

Holzmann, M. and Pawlowski, J.: An updated classification of rotaliid foraminifera based on ribosomal DNA phylogeny, Marine Micropaleontology, 132, 18–34, http://dx.doi.org/10.1016/j.marmicro.2017.04.002, 2017.

Kadan, Y., Aram, L., Shimoni, E., Levin-Zaidman, S., Rosenwasser, S., and Gal, A.: In situ electron microscopy characterization of intracellular ion pools in mineral forming microalgae, Journal of Structural Biology, 210, 107465, https://doi.org/10.1016/j.jsb.2020.107465, 2020.

Kampschulte, A., Bruckschen, P., and Strauss, H.: The sulphur isotopic composition of trace sulphates in Carboniferous brachiopods: implications for coeval seawater, correlation with other geochemical cycles and isotope stratigraphy, Chemical Geology, 175, 149-173, https://doi.org/10.1016/S0009-2541(00)00367-3, 2001.

Kester, D. R., Duedall, I. W., Connors, D. N., and Pytkowicz, R. M.: Preparation of artificial seawater 1, Limnology and oceanography, 12, 176-179, https://doi.org/10.4319/lo.1967.12.1.0176, 1967.

Kitano, Y. and Hood, D. W.: Calcium carbonate crystal forms formed from sea water by inorganic processes, Journal of the Oceanographical Society of Japan, 18, 141-145, https://doi.org/10.5928/kaiyou1942.18.141, 1962.

https://doi.org/10.5194/egusphere-2023-631
Preprint. Discussion started: 6 April 2023

Kitano, Y., Okumura, M., and Idogaki, M.: Incorporation of sodium, chloride and sulfate with calcium carbonate, Geochemical Journal, 9, 75-84, https://doi.org/10.2343/geochemj.9.75, 1975.

Kontrec, J., Kralj, D., Brečević, L., Falini, G., Fermani, S., Noethig-Laslo, V., and Mirosavljević, M.: Incorporation of Inorganic Anions in Calcite, EurJIC, 2004, 4579-4585, https://doi.org/10.1002/ejic.200400268, 2004.

Langer, M. R.: Biosynthesis of glycosaminoglycans in foraminifera: A review, Marine Micropaleontology, 19, 245-255, 1992.

Langer, M. R.: Assessing the contribution of foraminiferan protists to global ocean carbonate production, The Journal of Eukaryotic Microbiology, 55, 163-169, https://doi.org/10.1111/j.1550-7408.2008.00321.x, 2008.

Laakso, T. A., Waldeck, A., Macdonald, F. A., and Johnston, D.: Volcanic controls on seawater sulfate over the past 120 million years, PNAS, 117, 21118–21124, https://doi.org/10.1073/pnas.1921308117, 2020.

Le Calvcz, J.: Recherches sur les foraminifères. 1. Développernent et reproduction. Archives Zoologie Expérimentale et Génerale 80, 163-333, 1938.

Le Houedec, S., Erez, J., and Rosenthal, Y.: Testing the influence of changing seawater Ca concentration on Elements/Ca ratios in planktic foraminifera: A culture experiment, Geochemistry, Geophysics, Geosystems, 22, e2020GC009496, https://doi.org/10.1029/2020GC009496, 2021.

Lemelle, L., Bartolini, A., Simionovici, A., Tucoulou, R., De Nolf, W., Bassinot, F., and de Garidel-Thoron, T. : Nanoscale trace metal imprinting of biocalcification of planktic foraminifers by Toba's super-eruption, Scientific Reports, 10, 10974, https://doi.org/10.1038/s41598-020-67481-w, 2020.

Lin, C. Y., Turchyn, A. V., Steiner, Z., Bots, P., Lampronti, G. I., and Tosca, N. J.: The role of microbial sulfate reduction in calcium carbonate polymorph selection, Geochimica et Cosmochimica Acta, 237, 184-204, https://doi.org/10.1016/j.gca.2018.06.019, 2018.

Lowenstein, T. K., Timofeeff, M. N., Brennan, S. T., Hardie, L. A., and Demicco, R.V.: Oscillations in Phanerozoic seawater chemistry: Evidence from fluid inclusions, Science 294, DOI: 10.1126/science.1064280, 2001.

Lowenstein, T. K., Hardie, L. A., Timofeeff, M. N., and Demicco, R. V.: Secular variation in seawater chemistry and the origin of calcium chloride basinal brines, Geology, 31, 857-860, https://doi.org/10.1130/G19728R.1, 2003.

Marenco, P. J., Corsetti, F. A., Kaufman, A. J., and Bottjer, D. J.: Environmental and diagenetic variations in carbonate associated sulfate: an investigation of CAS in the Lower Triassic of the western USA, Geochimica et Cosmochimica Acta, 72, 1570-1582, https://doi.org/10.1016/j.gca.2007.10.033, 2008.

Nagai, Y., Uematsu, K., Chen, C., Wani, R., Tyszka, J., and Toyofuku, T.: Weaving of biomineralization framework in rotaliid foraminifera: implications for paleoceanographic proxies, Biogeosciences, 15, 6773-6789, https://doi.org/10.5194/bg-15-6773-2018, 2018.

de Nooijer, L. J., Toyofuku, T., and Kitazato, H: Foraminifera promote calcification by elevating their intracellular pH, Proc. Natl Acad. Sci., 106, 15374–15378, 2009.

de Nooijer, L. J., Spero, H. J., Erez, J., Bijma, J., and Reichart, G. J: Biomineralization in perforate foraminifera, Earth-Science Reviews, 135, 48-58, https://doi.org/10.1016/j.earscirev.2014.03.013, 2014.

Paris, G., Sessions, A. L., Subhas, A. V., and Adkins, J. F.: MC-ICP-MS measurement of $\delta^{34}S$ and $\Delta^{33}S$ in small amounts of dissolved sulfate, Chemical Geology 345, 50–61, http ://dx.doi.org/10.1016/j.chemgeo.2013.02.022, 2013.

Paris, G., Fehrenbacher, J. S., Sessions, A. L., Spero, H. J., and Adkins, J. F.: Experimental determination of carbonate-associated sulfate $\delta^{34}S$ in planktonic foraminifera shells. Geochem., Geophys., Geosyst., 15, 1452-1461, doi:10.1002/2014GC005295, 2014.

Paytan, A., Kastner M., Campbell, D., and Thiemens, M. H.: Sulfur Isotopic Composition of Cenozoic Seawater Sulfate, Science 282, 1459-1462, DOI: 10.1126/SCIENCE.282.5393.145, 1998.

https://doi.org/10.5194/egusphere-2023-631
Preprint. Discussion started: 6 April 2023

Perrin, J., Rivard, C., Vielzeuf, D., Laporte, D., Fonquernie, C., Ricolleau, A., and Floquet, N.: The coordination of sulfur in synthetic and biogenic Mg calcites: The red coral case, Geochimica et Cosmochimica Acta, 197, 226-244, https://doi.org/10.1016/j.gca.2016.10.017, 2017.

Present, T. M., Paris, G., Burke, A., and Fischer, W. W.: Large carbonate associated sulfate isotopic variability between brachiopods, Earth and Planetary Science Letters, 432, 187-198, https://doi.org/10.1016/j.epsl.2015.10.005, 2015.

Present, T. M., Adkins, J. F., and Fischer, W. W.: Variability in sulfur isotope records of Phanerozoic seawater sulfate, Geophysical Research Letters, 47, e2020GL088766, https://doi.org/10.1029/2020GL088766, 2020.

Rennie, V. C. and Turchyn, A. V.: The preservation of δS^{34}_{SO4} and δO^{18}_{SO4} in carbonate-associated sulfate during marine diagenesis: A 25 Myr test case using marine sediments, Earth and Planetary Science Letters, 395, 13-23, https://doi.org/10.1016/j.epsl.2014.03.025, 2014.

Rennie, C. F., Paris, G., Sessions, A. L., Abramovich, S., Turchyn, A. V., and Adkins, J. F.: Cenozoic record of $\delta^{34}S$ in foraminiferal calcite implies an early Eocene shift to deep-ocean sulfide burial, Nature Geoscience, 11, 761-765, https://doi.org/10.1038/s41561-018-0200-y, 2018.

Richardson, J. A., Newville, M., Lanzirotti, A., Webb, S. M., Rose, C. V., Catalano, J. G., and Fike, D. A.: The source of sulfate in brachiopod calcite: Insights from μ-XRF imaging and XANES spectroscopy, Chemical Geology, 529, 119328, https://doi.org/10.1016/j.chemgeo.2019.119328, 2019.

Rollion-Bard, C. and Erez, J.: Intra-shell boron isotope ratios in the symbiont-bearing benthic foraminiferan Amphistegina lobifera: Implications for $\delta^{11}B$ vital effects and paleo-pH reconstructions, Geochimica et Cosmochimica Acta, 74, 1530-1536, https://doi.org/10.1016/j.gca.2009.11.017, 2010.

Sabbatini, A., Bédouet, L., Marie, A., Bartolini, A., Landemarre, L., Weber, M. X., Kade Mahardika, I. G. N., Berland, S., Zito, F., Vénec-Peyré, M. T.: Biomineralization of *Schlumbergerella floresiana*, a significant carbonate-producing benthic foraminifer, Geobiology 12, 289-307, https://doi.org/10.1111/gbi.12085, 2014.

Sandberg, P. A.: An oscillating trend in Phanerozoic non-skeletal carbonate mineralogy, Nature, 305, 19-22, 1983.

Schiebel, R.: Planktic foraminiferal sedimentation and the marine calcite budget, Global Biogeochemical Cycles, 16, 1065, https://doi.org/10.1029/2001GB001459, 2002.

Schmidt, S., Hathorne, E. C., Schönfeld, J., and Garbe-Schönberg, D.: Heavy metal uptake of nearshore benthic foraminifera during multi-metal culturing experiments, Biogeosciences, 19, 629–664, https://doi.org/10.5194/bg-19-629-2022, 2022.

Tamenori, Y., Yoshimura, T., Luan, N. T., Hasegawa, H., Suzuki, A., Kawahata, H., and Iwasaki, N.: Identification of the chemical form of sulfur compounds in the Japanese pink coral (*Corallium elatius*) skeleton using μ-XRF/XAS speciation mapping, Journal of Structural Biology, 186, 214-223, https://doi.org/10.1016/j.jsb.2014.04.001, 2014.

Tamenori, Y., and Yoshimura, T.: Sulfur speciation in growth layers of shell cross section of the long-lived bivalve *Margaritifera laevis* using synchrotron spectromicroscopy analysis, Geochimica et Cosmochimica Acta, 237, 357-369, https://doi.org/10.1016/j.gca.2018.07.002, 2018.

Toyofuku, T., Matsuo, M. Y., de Nooijer, L. J., Nagai, Y., Kawada, S., Fujita, K., Reichart, G.-J., Nomaki, H., Tsuchiya, M., Sakaguchi, H., and Kitazato, H.: Proton pumping accompanies calcification in foraminifera, Nature Communications 8, 14145, DOI: 10.1038/ncomms14145, 2017.

Vachard, D., Pille, L., and Gaillot, J.: Palaeozoic Foraminifera: Systematics, palaeoecology and responses to global changes, Rev. de Micropaleontol., 53, 209–254, https://doi.org/10.1016/j.revmic.2010.10.001, 2010.

Van Dijk, I., de Nooijer, L. J., and Reichart, G. J.: Trends in element incorporation in hyaline and porcelaneous foraminifera as a function of pCO_2, Biogeosciences, 14, 497-510, doi:10.5194/bg-14-497-2017, 2017.

https://doi.org/10.5194/egusphere-2023-631
Preprint. Discussion started: 6 April 2023

Van Dijk, I., Barras, C., De Nooijer, L. J., Mouret, A., Geerken, E., Oron, S., and Reichart, G. J.: Coupled calcium and inorganic carbon uptake suggested by magnesium and sulfur incorporation in foraminiferal calcite, Biogeosciences, 16, 2115-2130, https://doi.org/10.5194/bg-16-2115-2019, 2019.

555 Weiner, S. and Erez, J.: Organic matrix of the shell of the foraminifer, *Heterostegina depressa*, The Journal of Foraminiferal Research, 14, 206-212, https://doi.org/10.2113/gsjfr.14.3.206, 1984.

https://doi.org/10.5194/egusphere-2023-631
Preprint. Discussion started: 6 April 2023

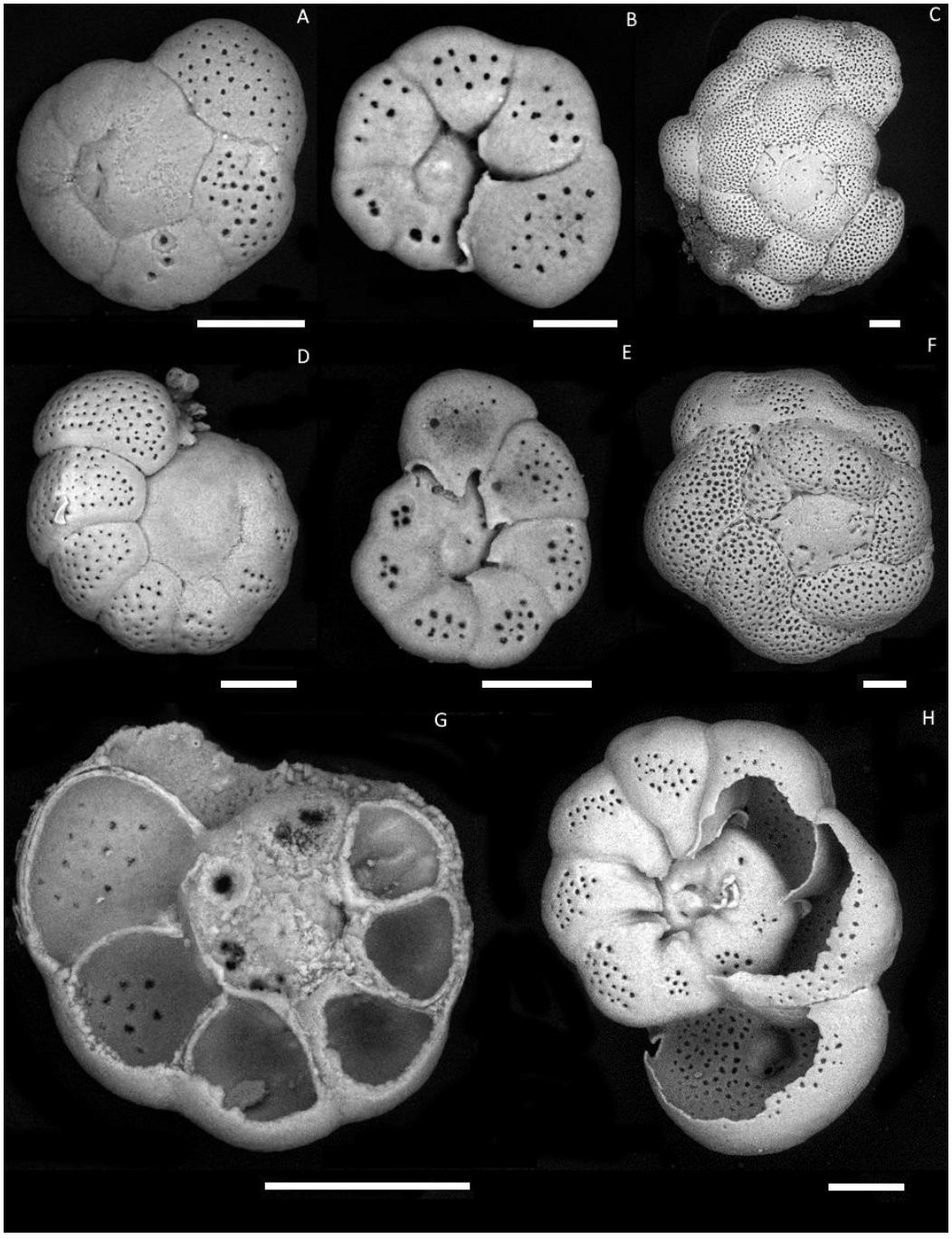

Figure 1. Foraminiferal strains cultured in this study. For1C1: (A) *Rosalina* like morphotype (11-12 chambers) reproducing asexually, dorsal view, (B) same as A, ventral view, (C) Morphotype with more than 12 chambers, starting as *Rosalina* morphotype and then developing annular disposition of the last chambers, dorsal view. C1Tg: (D) *Rosalina* like morphotype, (E) same as D, ventral view, (F) morphotype with annular arrangement of the last chambers, dorsal view. (G and H) ventral view of C1Tg with a broken test permitting to see the layered structure of the test's wall (G) and the foramen position inside of the test (H). Scale bar 50 µm, SEM picture in BSE mode operated at 10 to 22 mPa and 20 000kV.

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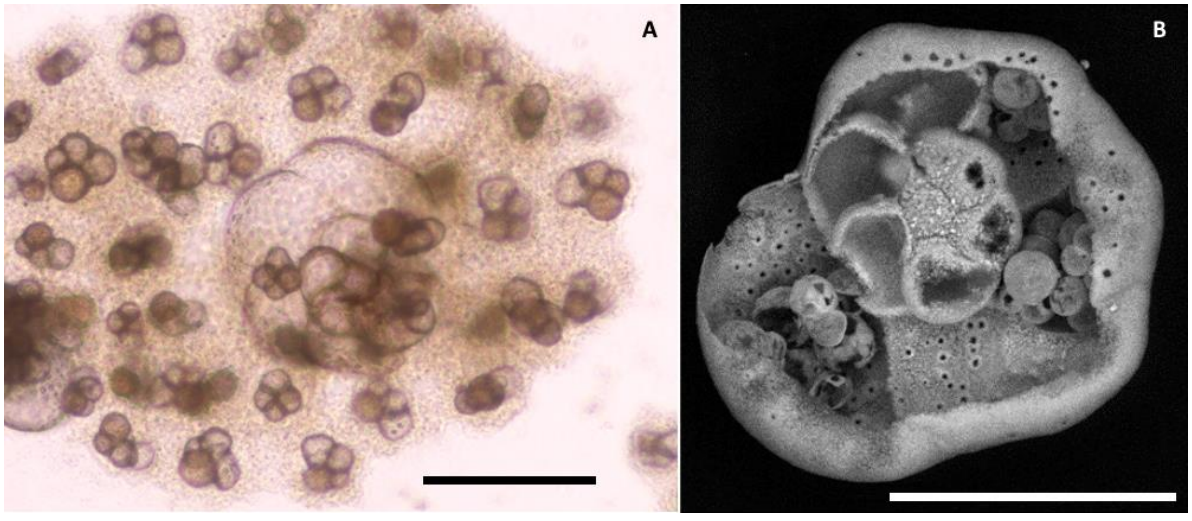

Figure 2. Asexual reproduction of an individual of the For1C1 strain. (A) Light microscope image of a megalospheric schizont adult that has ~12 visible chambers, and whose cell has divided asexually into viable juveniles (for further detail, see Appendix A). The darker appearance of the juveniles compared to the adult is due to the presence of cellular material. After division, the adult is empty and its test partially dissolved, as shown in the SEM micrograph (B). Scale bar 100 µm.

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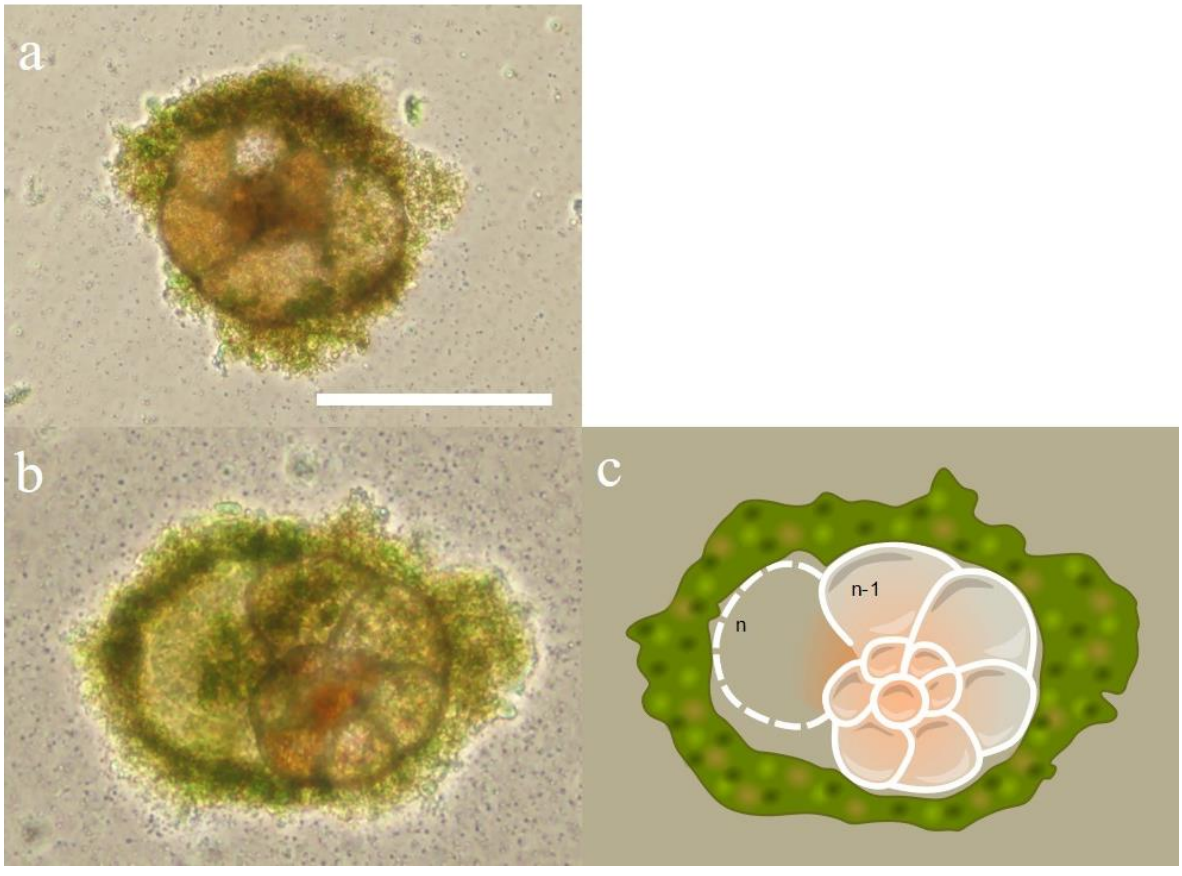

Figure 3. Chamber formation during day 2 of the set 1 series of experiment. Two For1C1 individuals (from Banyuls) in (a) ASW[5] (containing 5 mM SO_4^{2-}) and (b) ASW[10] (containing 10 mM SO_4^{2-}) and its schematic representation illustrating the new chamber (n) formation and the surrounding gangue (algal cyst) constituted by the foraminifer by the accumulation of foreign detritus and other materials, confining the new chamber in formation in a microenvironment. In the case of rotaliid foraminifera, the formation of a new chamber begins with the isolation of the chamber volume from the surrounding environment by a structure which probably form the organic scaffolding that shapes the morphology of the chamber and serves as a template for the calcification of the wall (Bé et al., 1979; De Noojier et al., 2014; Nagai et al., 2018). Precipitation of calcium carbonate takes place on both sides of an organic layer, called primary organic sheet (POS, Erez, 2003), sandwiched between the outer and inner organic layers. Scale bar 100 µm.

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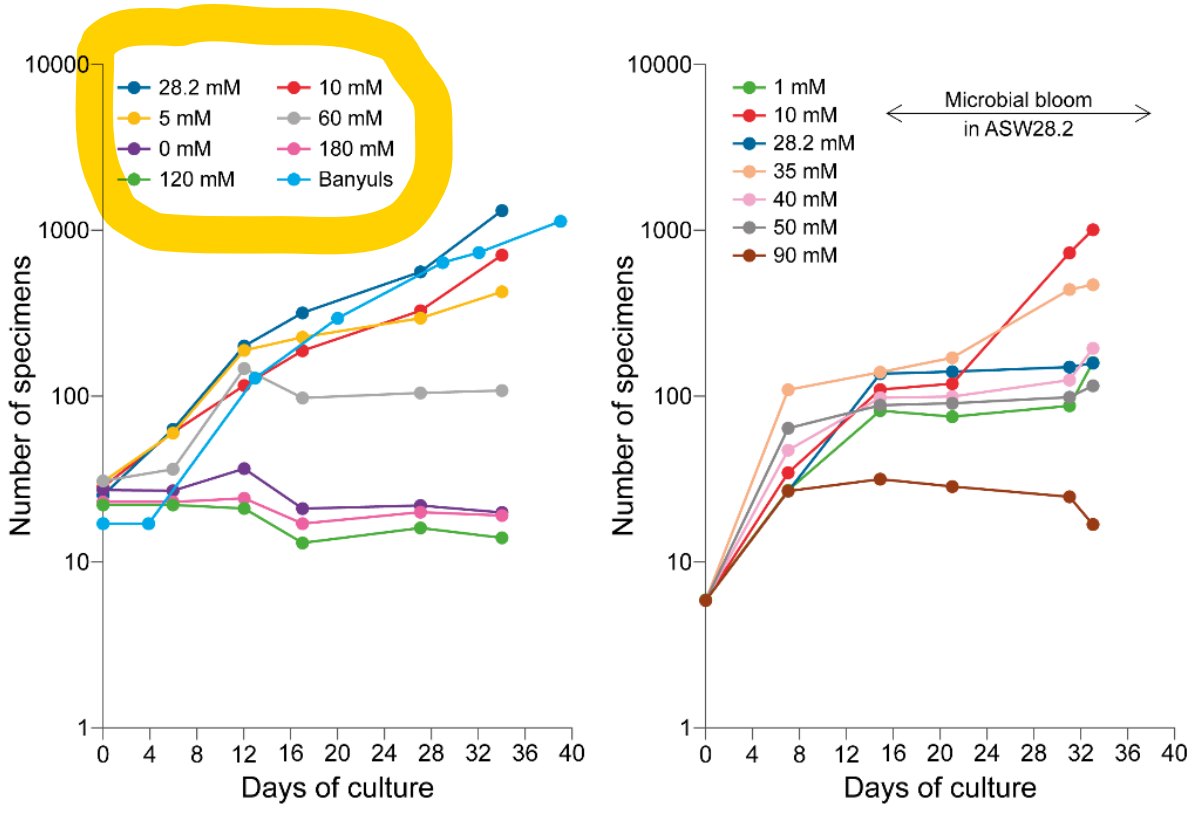

Figure 4. Evolution of the number of the attached specimens of the For1C1strain in each culture medium at different [SO_4^{2-}] for (a) Set 1 and (b) Set 2. The number of foraminifera counted (with a precision of ± 3 individual) corresponds to the living ones adhering to the bottom of the Petri dish before the change of medium. The increase in the number of individuals is due to asexual multiplication (see Appendix A). In Set 1, the largest population in terms of size occurs for 28.2 mM (ASW[28] and NSW Banyuls). In Set 2, a microbial bloom occurred after 12 days in medium ASW[28], likely affecting the reproduction rate (Appendix C, Appendix Fig. C1). The y-axes are on log scales.

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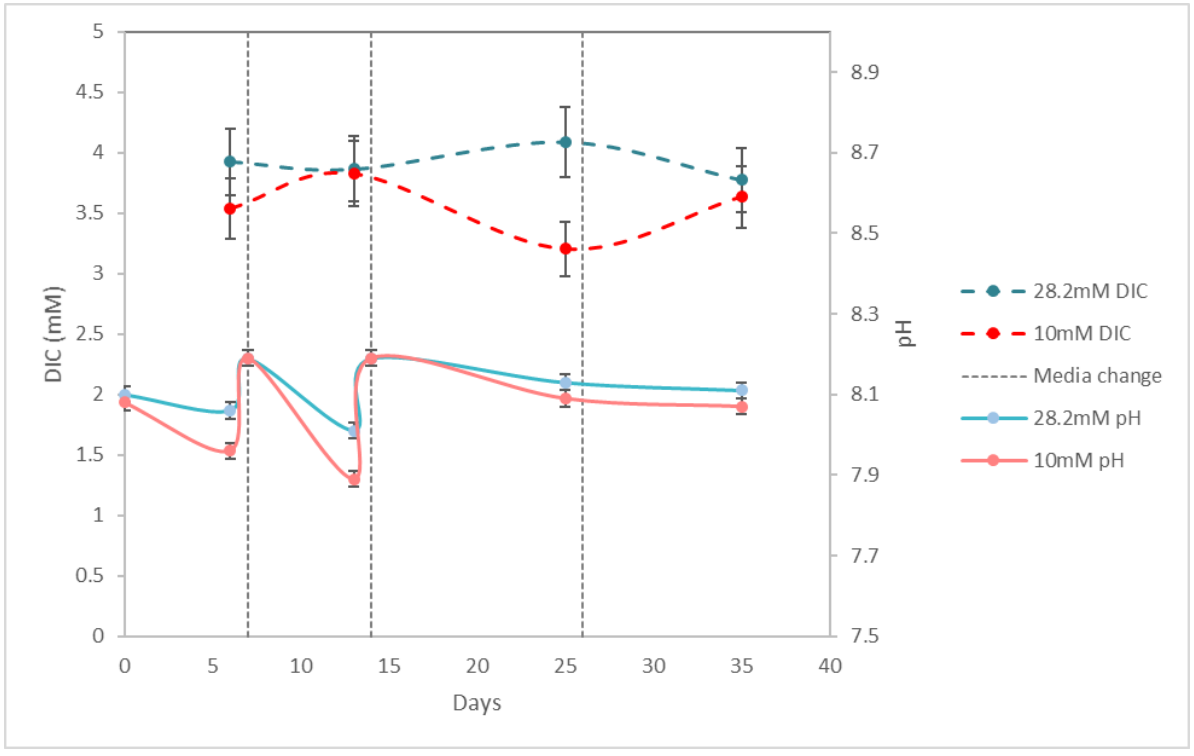

Figure 5. Evolution of pH and DIC over 35 days of For1c1 culture at 10 mM and 28.2 mM during SET2, with a change of medium on days 7, 14 and 26. Measured DIC in all experiments (Table 3) varies between 3.2 mM and 4.1 mM, it decreases by calcite formation and increases by specimen respiration. The variation of pH in all experiments when measured (Table 2) is between 7.89 and 8.19. The decrease in pH is caused by both respiration (CO_2 production) and calcite precipitation.

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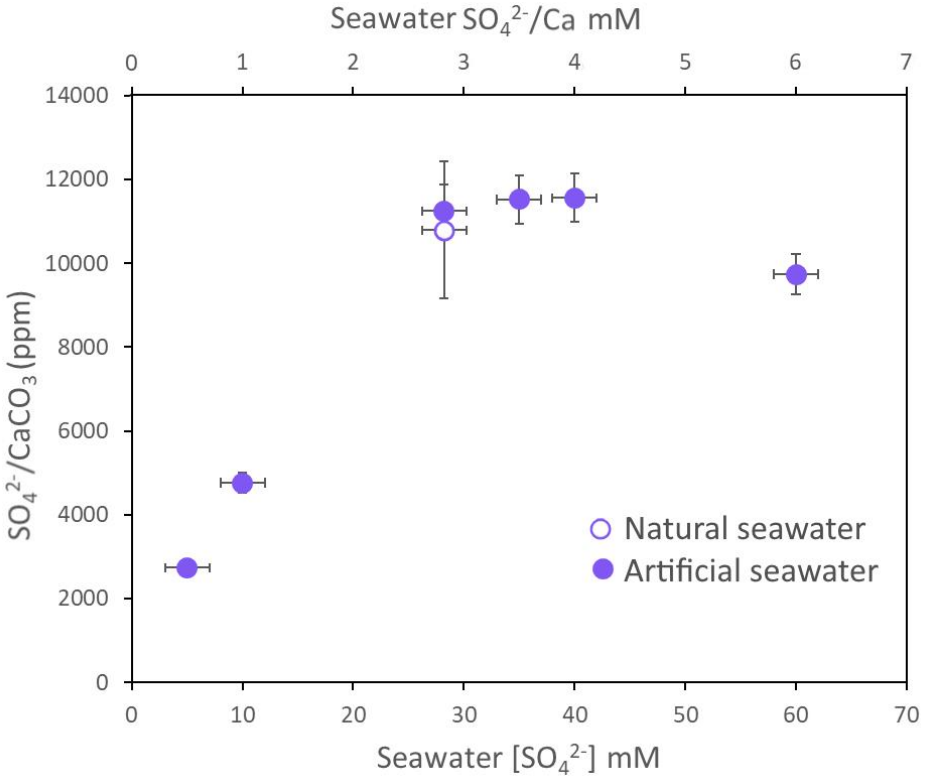

Figure 6. $SO_4^{2-}/CaCO_3$ ratio on tests of the For1C1 strain at the end of SET1 and SET2 experiments, as a function of seawater $[SO_4^{2-}]$ (5, 10, 28.2, 35, 40 and 60 mM). Each measurement has been performed on a pool of hundred to several hundreds of specimens.

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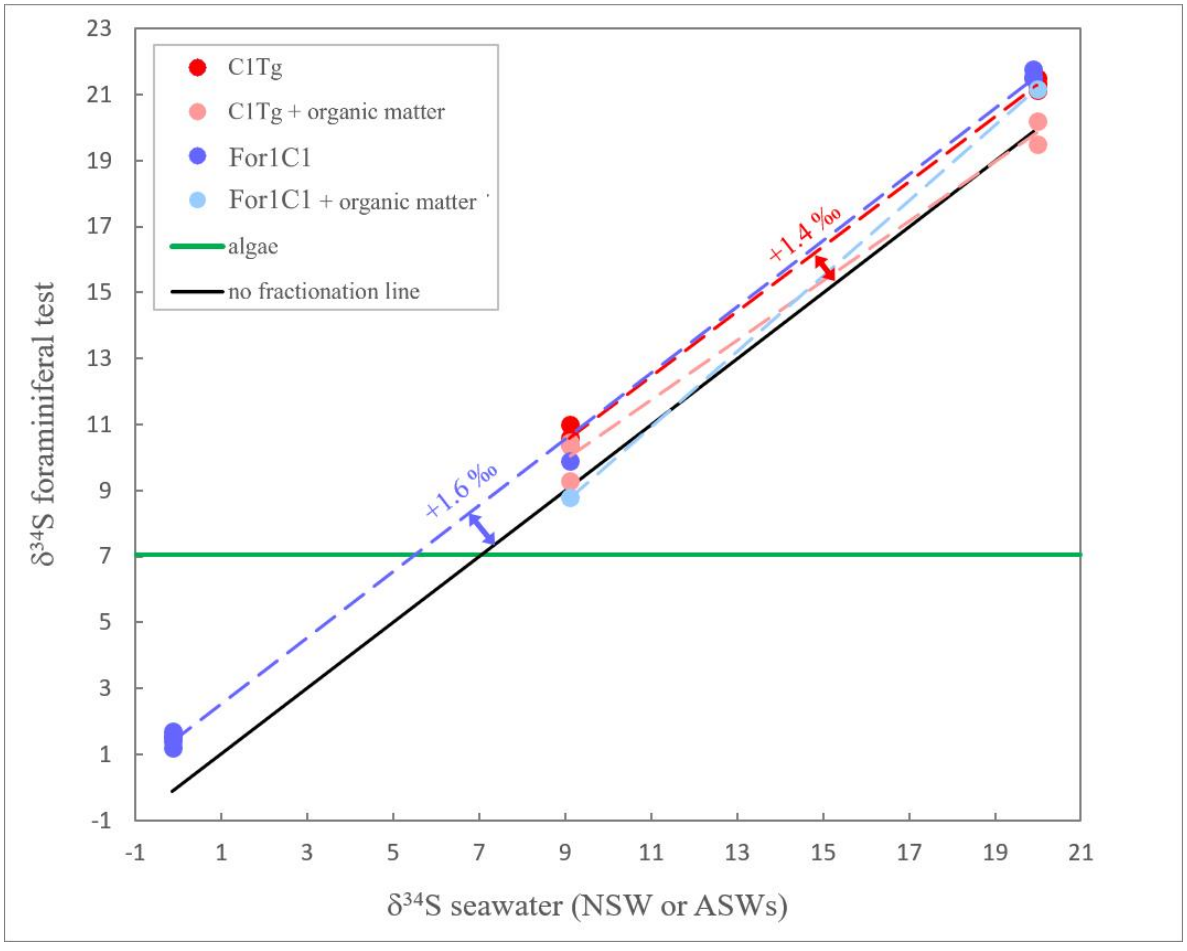

Figure 7. $\delta^{34}S$ in foraminiferal test (with or without organic matter) for the two strains For1C1 and C1Tg. The two strains were cultured in several media (For1C1 in ASW[5], ASW[10] ASW[28], ASW[35], ASW[40], ASW[60] and NSW (Banyuls), C1Tg in NSW (Concarneau) and ASW[28]) whose $\delta^{34}S$ has been also measured. The $\delta^{34}S$ value of the media depend on the salts used to make the solution. The green line corresponds to the $\delta^{34}S$ composition of the algae that were fed to the foraminifera, and whose isotopic composition remain stable. 2σ error bars (±0.2‰ to ±0.3‰) are smaller than symbols.

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Table 1. Description of Set1 and Set2 experiments

Strains	Acclimation	Monitored culture experiments
For1C1 (maintained in cultures in NSW(Banyuls) for years	NSW(Banyuls)	Set 1: NSW(Banyuls)
	ASW[28]	Set 1: ASW[0], ASW[5], ASW[10], ASW[28], ASW[60], ASW[120], ASW[180]
		Set 2: ASW[1], ASW[10],ASW[28], ASW[35], ASW[40], ASW50, ASW[90]
C1TG (maintained in cultures in NSW(Concarneau) for years	NSW(Concarneau)	
	ASW[28]	

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610 **Table 2. pH values measured in media all along SET1 and SET2 experiments.**

Set 1								
Day	**ASW[0]**	**ASW[5]**	**ASW[10]**	**ASW[28]**	**ASW[60]**	**ASW[120]**	**ASW[180]**	**Banyuls**
0	8.09	8.09	8.08	8.1	8.1	8.14	8.14	nd
6	7.94	7.94	7.96	8.06	7.97	7.94	8.02	nd
7	8.17	8.19	8.19	8.19	8.19	8.17	8.2	nd
13	7.89	7.89	7.89	8.01	7.93	7.83	8.02	8.06*
14	8.17	8.19	8.19	8.19	8.19	8.17	8.2	nd
25	8.1	8.1	8.09	8.13	8.15	8.16	8.2	8.07*
35	8.05	8.07	8.07	8.11	8.12	8.14	8.16	8.03*

Set 2							
Day	**ASW[1]**	**ASW[10]**	**ASW[28]**	**ASW[35]**	**ASW[40]**	**ASW[50]**	**ASW[90]**
7	8.1	8.12	8.14	8.09	8.12	8.12	8.17
16	8.08	8.12	8.15	8.15	8.14	8.13	8.14

***: Foraminiferal culture in seawater from Banyuls started with a delay, making the pH measurements day 12, 22 and 26**

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Table 3. DIC concentration in culture media all along Set 1 and Set 2 experiments

Days	6	12	13	14	22	25	29	35	38	46	52	53
Media	DIC mM +/- 4%											
ASW[1]									3.5			
ASW[5]				4.1								
ASW[10]	3.5		3.8			3.2		3.6				3.7
ASW[28]	3.9		3.9			4.1		3.8				3.7
NSW		3.5			3.7		3.8					
ASW[35]									3.6	3.8	3.7	
ASW[40]									3.6			
ASW[50]									3.5			
ASW[60]						4.1						
ASW[90]											3.9	

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Table 4. Sulfate concentration and isotopic composition measured in the foraminiferal calcite

Species	Media	$SO_4/CaCO_3$	$\Delta^{34}S$*	$\delta^{34}S$ CAS	$\delta^{34}S$ SO_4^{2-} water	$[SO_4^{2-}]$ media
		ppm +/- 5%	±0.2‰	±0.2‰	±0.2‰	(mM)
For1C1	ASW[5]	2740	1.7‰	1.6‰	-0.1‰	5
For1C1	ASW[10]	4760	1.5‰	1.4‰	-0.1‰	10
For1C1	ASW[28]	11700	Nd	Nd	Nd	28.2
For1C1	ASW[28]	10800	0.8‰	9.9‰	9.1‰	28.2
For1C1	NSW	11900	1.9‰	21.8‰	19.9‰	28.2
For1C1	NSW	11600	1.6‰	21.5‰	19.9	28.2
For1C1	NSW	8910	1.7‰	21.5‰	19.9	28.2
For1C1	ASW[35]	11500	1.6‰	1.5‰	-0.1	35
For1C1	ASW[40]	11600	1.3‰	1.2‰	-0.1	40
For1C1	ASW[60]	9740	1.8‰	1.7‰	-0.1	60
For1C1 + org	NSW	16900	1.2‰	21.2	20.0	28.2
For1C1 + org	ASW[28]	8130	-0.3‰	8.8	9.1	28.2
C1Tg	NSW	6990	1.5‰	21.5	20.0	28.2
C1Tg	NSW	7130	1.4‰	21.3	20.0	28.2
C1Tg	NSW	6530	1.2‰	21.2	20.0	28.2
C1Tg	NSW	13130	1.3‰	21.3	20.0	28.2
C1Tg	NSW	11250	1.2‰	21.1	20.0	28.2
C1Tg	ASW[28]	6080	1.3‰	10.4	9.1	28.2
C1Tg	ASW[28]	9000	1.5‰	10.6	9.1	28.2
C1Tg	ASW[28]	8710	1.3‰	10.4	9.1	28.2
C1Tg	ASW[28]	nd	1.9‰	11.0	9.1	28.2
C1Tg + org	NSW	26420	-0.5‰	19.5	20.0	28.2
C1Tg + org	NSW	17460	0.2‰	20.2	20.0	28.2
C1Tg + org	ASW[28]	8110	1.3‰	10.4	9.1	28.2
C1Tg + org	ASW[28]	6940	1.4‰	10.5	9.1	28.2
C1Tg + org	ASW[28]	10400	0.2‰	9.3	9.1	28.2

*$\Delta^{34}S = \delta^{34}S$ CAS - $\delta^{34}S$ SO_4^{2-} water

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6 Appendices

6.1 Appendix A

Foraminifer taxonomy

The two selected strains come from two distinct locations, from Banyuls (Mediterranean Sea) and Concarneau (Atlantic Ocean). Morphologically they may be related to the family Rosalinidae (Holzmann and Pawlowski, 2017). They are attached forms with a low trochospiral hyaline calcitic perforate test, with a peripherical low arch aperture on the umbilical side bordered by lips (Fig. 1). Chamber interior is simple (Fig. 1). Two morphotypes are noticeable in both the strains (Fig. 1). Individuals usually reproducing asexually after every 12-15 days when their test reaches a development of 11-12 chambers (Fig. 2), are morphologically very close to the genus *Rosalina* (Fig. 1). Individuals who lived for several weeks adding more than 12 chambers have the last chambers with an annular arrangement (Fig. 1).

Individuals with a *Rosalina* like morphology (Fig. 1) probably belong to the schizont generation of their trimorphic life cycle (alternating gamont-agamont-schizont-gamont generations), documented for example in *Planorbulina mediterranenis* and a few dozen other species (Le Calvez, 1938; Dettmering et al., 1998). More precisely, they are diploid megalospheric schizonts that have entered a cycle of successive asexual reproduction (apogamic cycle) (Fig. 2), during which the new generation of schizonts is produced by schizogony, i.e. by multiple fission of a multinucleate parental cytoplasm (Le Calvez, 1938; Dettmering et al., 1998). For this reason, it is not obvious to identify them morphologically at the species level because the morphology of the diploid agamont microspheric and/or of the haploid megalospheric gamont parent generation, on which the description of the species has often been made, is unknown to the best of our knowledge. For now, we leave these forms in open nomenclature and call them by the name of the strains For1C1 and C1Tg. Adult specimens of these strains are smaller than the traditional foraminifer fraction obtained after sieving (through >125 µm mesh) in geochemical studies, they thus may be common, while rarely collected because of their size.

6.2 Appendix B

Table B1. Weekly number of accumulated live individuals for each medium at different [SO_4^{-2}]

SET 1		Seawater [SO_4^{2-}]							
		0	**5**	**10**	**NSW**	**28.2**	**60**	**120**	**180**
Date	**Days**	**Number of specimens**							
23/03/2018	0	27	30	28	17	25	31	22	23
29/03/2018	6	27	60	61	17	63	36	22	23
04/04/2018	12	36	188	116	128	199	151	21	24
09/04/2018	17	21	227	187	294	317	98	13	17
18/04/2018	27	22	293	322	638	556	104	16	20
25/04/2018	34	20	425	713	732	1312	108	14	19

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SET 2		Seawater [SO_4^{2-}]						
		1	**10**	**28.2**	**35**	**40**	**50**	**90**
Date	**Days**	**Number of specimens**						
07/05/2018	0	6	6	6	6	6	6	6
14/05/2018	7	27	35	27	109	48	64	27
22/05/2018	15	82	109	138	141	97	89	32
28/05/2018	21	76	120	142	173	98	91	29
06/06/2018	31	87	737	151	444	127	97	25
08/06/2018	33	161	1014	159	470	194	117	17

Table B2. Sulfur isotope composition of media and algae cells

Sample	**$\delta^{34}S$ +/- 0.2**
NSW Banyuls before culture	21.1
NSW after feeding For1C1	19.9
ASW[28] before culture	9.1
ASW[28] after feeding For1C1	9.1
ASW[28] after feeding C1Tg	9.2
ASW(all used concentrations except 28) after feeding For1C1*	-0.1
Algae media	5.4
Algae cells	7.0

* Different salts were used to make all ASW and ASW28

6.3 Appendix C

Potential experimental bias

We designed Set 2 to replicate the ASW[28] and ASW[10] results as well as to extend the range of concentrations and ran it right after Set 1 (see methods for more details). Two differences can be observed. First the reproduction rate is significantly higher in the ASW[10] media of Set 2 than it was in Set 1 even though the media were identical. However, it might be related to the starting number of foraminifera for each experiment, in Set 2 we started each culture experiment with 6 foraminifera individuals (instead of 28 as in Set 1), each 6 individuals were chosen more carefully, which could induce a bias and explain a more active behavior during experiment 2. If it were the case, the bias would nonetheless be systematic and similar for each medium (all Petri dishes in set 2 started with 6 forams) and thus do not prevent comparison of results within set 2.

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The second difference is observed in the ASW[28] medium in set 2. The reproduction rate, which was the highest observed, 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 (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.

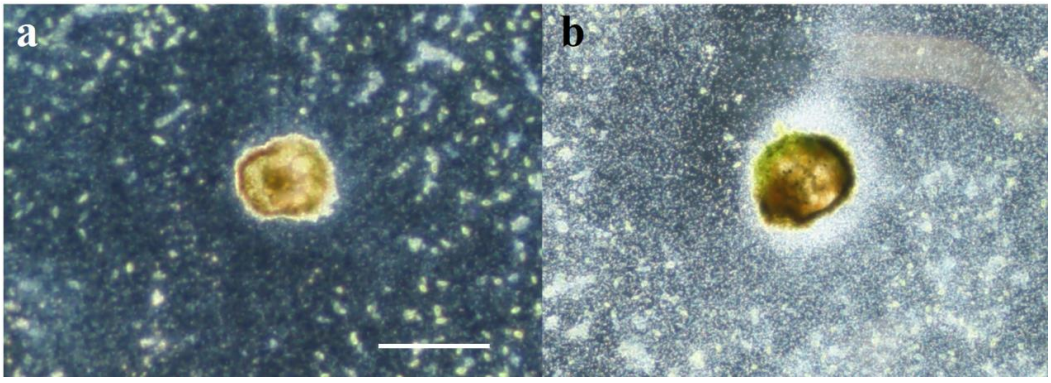

Figure C1. Optical microscopy imaging in dark field, the foraminifera are observed from below, the back ground appears black, algae greenish, and bacterial contamination cloudy white. (a) Foraminifera in ASW[10] during experiment 2 where microbial spread stays limited. (b) Foraminifera in ASW[28] during experiment 2 where microbial bloom was uncontrolled after 15 days and could not be reduced.