

## Short Comment 1, Inge van Dijk

Thank you for presenting for the first time data on the distribution of F and Cl in foraminiferal calcite. I have some short comments of issues I noticed during a quick read of the manuscript, which are mainly concerning the lack of details of the culture experiments and the graphical presentation of the data. I leave a proper review to the invited referees.

Thank you for the feedback. We will provide further details on the culture experiments and materials used.

### Comment SC1.1

Fig 1. The miliolid species come from two salinity conditions, according to table 1. From which salinities are the specimens show in Fig. 1? And which chambers: ultimate, penultimate, etc?

Answer: The salinities for the specimens shown in Figure 1 were presented in Table 1. As this study does not focus on salinity, we decided not to report salinities (and other environmental parameters) in the figure or figure caption, but instead to provide an overview in Table 1. We agree that the information in Table 1 could only be linked to Figure 1 via Fig. A1, which was suboptimal.

Changes: To facilitate identification, we added specimen numbers to Figure 1 and we explicitly mention in the figure caption that details on the specimens used are presented in Table 1. Chamber numbers (F, F-1, etc.) were added to Fig. A1.

### Comment SC1.2

I think a SEM picture of the studied areas would be a good addition to Fig. 1. I see the general overview pictures in the appendix, but I would like to see also the higher magnification image.

Answer: Figure 1 is already rather complex and we rather refrain from adding even more complexity to it. The context of the detailed nanoSIMS images are provided by SEM images shown in Fig. A1. These are high resolution images that can be zoomed in by the reader.

### Comment SC1.3

Looking at the location of the measurements of the miliolids, and the explanation of the culture set-up, how can you assure the measurements were done on newly formed (experimental) calcite?

Judging the orientation of the foraminifera in the SEM images in Appendix A1, it seems like you are not measuring e.g. the last chambers, which are a bit less complex. Especially in the case of *Archaias*, the last chambers seem to be on the top left of the image, and it looks like the authors choose a quite complex location for the analysis. Why not analyse the last chambers, where the direction of growth is more clear? Also, the polishing of the *Sorites* doesn't seem to include the last chambers, because they appear to be still inside the resin (or was the specimen broken?).

Answer: The cultures were started with juvenile specimens, possessing 2-3 chambers at the start of the experiment. All additional chambers were formed during the course of the experiment. The miliolid species were cultured in media containing the fluorescent indicator calcein to identify newly formed calcite in retrospect. Positions for nanoSIMS imaging were carefully selected based on the quality of the surface preparation and position in the specimen. Where possible, distal chambers have been measured, but more proximal chambers were preferred in case their cross-sectional surfaces were flatter or cleaner.

Changes: The following information was added to the methods section: "The fields of view for NanoSIMS imaging were carefully selected using SEM images on the basis of the position in the specimen and the quality of the surface preparation. Where possible, distal chambers were measured, but more proximal chambers were preferred if their cross-sectional surfaces appeared flatter or cleaner."

### Comment SC1.4

Please indicate the chamber numbers (F, F-1 etc) and, most important, which ones are precipitated in the experiment. This is crucial, since the authors compare to the culture conditions in Fig. 3 and Fig. A4.

Answer: This comment relates to SC1.3, see also the answer to SC1.3. We assume this comment refers to the rotaliid species, where chambers are commonly indicated with F, F-1, etc.

Changes: We indicated chamber numbers in Fig. A1 for the rotaliid specimens.

#### **Comment SC1.5**

Also, please mirror the scalebar in these figures for readability.

Answer: Done.

Changes: Scale-bar text mirrored for better readability.

#### **Comment SC1.6**

In my opinion, the culture experiments have to be described more in detail, clearly stating the differences between the set-ups. Even though the other experiments are published already, some basic details can be stated in section 2.1. Also there is no clear indication how samples were cleaned, while the cleaning can have a major effect on the element distribution (Glock et al., 2019: <https://doi.org/10.3389/feart.2019.00175>). Digging through the publications of the other experiments, this information can be retrieved. But cleaning method is not presented for the unpublished experiment. Please add this information.

Answer: Done.

Changes: The following was added to the methods section: “The *A. angulatus* and *S. marginalis* specimens were collected in Sint Eustatius (Oranjestad Bay, 17.479751°N -62.987273°W). The culture experiments with *A. angulatus* and *S. marginalis* were conducted in the same manner as described in van Dijk et al. (2017), with the exception of media preparation. Culture media of different salinities were prepared by mixing natural 0.2µm filtered seawater with deionized water and 'instant ocean' salt, to obtain a range in salinities between 25-45. Calcein was added during the course of the experiment, and fluorescence images were used to identify newly precipitated calcite. The *A. lessonii* specimens are from Burger's Zoo, NL (van Dijk et al., 2019), with the culture conditions being reported in van Dijk et al. (2019). The specimens of *A. tepida* were collected on a tidal flat near Den Oever, the Wadden Sea, NL (Hayward et al., 2004), with the culture conditions being described in Geerken et al. (2018). For both the cultures of *A. lessonii* and *A. tepida*, 2-3 chambered juveniles were transferred into Petri dishes containing culture media with adjusted salinity and alkalinity, where the specimen precipitated additional chambers. Prior to embedding all specimens were cleaned using an adapted Barker protocol (Barker et al., 2013), only applying the organic removal/oxidation step, in which NaOH was replaced by NH<sub>4</sub>OH, as described in detail in Geerken et al. (2018).”

#### **Comment SC1.7**

How were the E/Ca measured for the miliolid species? Since these specimens are coming from an unpublished experiment, and are not “previously described (Geerken et al., 2018; van Dijk et al., 2019)”, as the authors state. Please give these details.

Answer: We meant the E/Ca ratios in the miliolid species were determined using the same methodology “as previously described [...]”.

Changes: for more clarity the text was adapted to “This was done by LA-ICP-MS for *A. tepida* and *A. lessonii* as previously described (Geerken et al., 2018; van Dijk et al., 2019). For *A. angulatus* and *S. marginalis* LA-ICP-MS analyses were performed using the same methodology as described in Geerken et al. (2018).”

#### **Comment SC1.8**

It looks like the *Archaias angulatus* cultured at salinity 40 has lower Na/Ca than the specimens from salinity of 30. What are the consequences for the Na/Ca – salinity proxy, and the idea that miliolids are precipitating from seawater vacuoles?

Answer: Large intra-specimen variability in Na/Ca has been shown for rotaliid species (e.g. Geerken et al., 2018). It may well be that miliolids exhibit even large intra-specimen Na/Ca variability as well, where a specimen cultured at salinity 40 can have a lower Na/Ca than a specimen cultured at a salinity of 30. To be able to draw any conclusions on what the consequences of individual specimen Na/Ca would be for the Na/Ca salinity proxy using miliolid species, further research is needed. This should involve culturing experiments using a statistically sound number of replicate specimen at a range of salinities.

Changes: As this manuscript focuses on the anions Cl and F, we have not included a note about the range in Na/Ca of the presented specimens.

#### **Comment SC1.9**

Also indicate the salinity conditions in the figures/captions in figures and tables, e.g. appendix A1, and table A1.

Answer: See answer to comment SC1.1.

Changes: We have added the specimen number where missing to facilitate finding the respective environmental conditions in Table 1.

#### **Comment SC1.10**

Consider changing the terminology, from rotaliid to hyaline and miliolid to porcelaneous.

Answer: We have considered different terminology, but chose for referring to the differences in terms of order instead of test appearance. We did so, because hyaline foraminifera also include globigerinids, which were not investigated in this study.

#### **Comment SC1.11**

For future work, please also consider to analyse also the natural chambers from the field for comparison with the experimental chambers. Especially for the specimens that were culture using Instant ocean salt, which is an industrial manufactured salt, lacking e.g. certain organic complexes.

Answer: a valuable suggestion for future work.

#### **Comment SC1.12**

Also, as indicated in van Dijk et al., 2019

(<https://www.frontiersin.org/articles/10.3389/feart.2019.00281/full>), there is a high intra- and inter-specimen variability for many elements. Therefore, please consider measuring several chambers and specimens to gain a robust dataset.

Answer: we are aware that a more robust data set is needed for drawing conclusions concerning proxy potential and relationships with environmental parameters. However, as we here present a pilot study into the spatial distribution of Cl and F in rotaliid vs. miliolid benthic foraminiferal species, we regard the current data set sufficient. We agree that future research using more replicates and consistent culturing conditions is needed to better understand the incorporation mechanisms and impact of environmental conditions on incorporation of Cl and F.

Changes: see also comment RC1.1. In our revised version, we have stated more clearly that the current data set does not allow for conclusions on proxy application.

## Referee Comments Anonymous Referee #1

Review for "Distribution of chlorine and fluorine in benthic foraminifera" by Roepert et al. This study looks at the incorporation of the anions chlorine and fluorine into the shells of benthic foraminifera. This has received almost no attention yet, although the conservative nature of these elements on the oceans would make them interesting to function as paleo proxies. Four different species, both rotaliid and miliolid, were cultured under controlled conditions. Analyses were performed using laser ablation and nanoSims. The distribution of chlorine and fluorine in the calcite varies between the low/intermediate-Mg species and the high-Mg species. Similar to other elements the lower Mg species show a clear banding of Cl and F related to the organic linings formed during biomineralization. As the biomineralization process is different in the high-Mg species in that no banding is developed, this is also not visible in the Cl and F content. So, the distribution of Cl and F depends on the biomineralization process and seems mostly connected to organic content. The manuscript is well written and organized, it is easy to read and extensive details on the methods are given. I do miss a few things on the methods though, and a final implications section or paragraph (see below). I recommend that this manuscript makes a valuable addition to Biogeosciences after minor revisions have been made.

We thank the referee for this constructive feedback. Details of our response are given below.

### Comment RC1.1

In the abstract the potential of these conservative elements as paleo-proxy is mentioned, but then apart from one sentence (Line 182) this is not coming back anymore. I suggest to include a final paragraph at the end of the discussion what these results imply for proxy development. Is it possible at all to conclude something about this? It is stated already that the number of samples and different setups is not large enough to identify trends, but could the extremely high-resolution also be an issue to determine their use as a proxy?

**Answer:** Our study did not aim at the development of a new proxy, but rather at exploring the incorporation of halogens. Consequently the data obtained do not allow to draw conclusions for proxy development. A more robust data set with, species-specific, replicated specimen per treatment would be needed. Also, large intra-test variability that is observed for many trace elements in foraminiferal shells using high-resolution imaging techniques implies replicate analyses on several chambers per specimen are necessary. Creating large data sets with replicate measurements on many specimens is not the strength of NanoSIMS and hence other analytical techniques are more suitable for studying potential proxy applicability. Still, our approach does provide distributional data of F and Cl so far not available.

**Changes:** We added a clearer statement to the discussion that potential proxy applicability of Cl/Ca and F/Ca cannot be evaluated based on our data.

### Comment RC1.2

For a commonly used proxy as Mg/Ca you also see a very heterogeneous distribution when looking at the micro-scale that does not appear to correlate with environmental conditions. But the actual proxy is the ratio that is representative for the whole shell (or enough laser profiles). So, how representative do you think your results are? Just six specimens on four different species, and a laser profile through each one showing how heterogeneous the distributions are, is not very much.

**Answer:** We acknowledge that our data set is limited to few fields of view, on a limited amount of specimens. However, lateral profiles have also been made for the fields of view where the images are not shown. The panels in Figure 1 show a representative image per species of those we analysed, and based on the similarity between the images within one species we do not expect appreciable differences in elemental distribution patterns if we imaged more fields of view. For the purpose of presenting key differences between rotaliid and miliolid species we consider our data sufficient. The data that are shown in Figures 3, 4 and A4 are averages of 1, 2 or 3 fields of view per

specimen including standard deviation. They resemble average elemental ratios as determined by LA-ICP-MS measurements, although, at a higher resolution. Since (in the case of the rotaliids) the field of view of the NanoSIMS images covered a cross-section through the shell wall, we expect the average of one NanoSIMS image to resemble an average LA-ICP-MS profile.

Changes: We have added “As such, lateral profiles that cover a representative fraction of a shell wall may be comparable to LA-ICP-MS profiles, albeit with a higher resolution.” to the methods section.

### **Comment RC1.3**

Section 2.1: More details on the culturing experiments are needed. Part of them are in Appendix B, but I think this would be much better to include into the main text.

Answer: This comment echoes that of Inge van Dijk and for a detailed response see answer to Comment SC1.6.

Changes: more details have been added in the main text, see answer to Comment SC1.6.

### **Comment RC1.4**

What I miss is on what part of the forams the analyses were done. I assume on the newly grown calcite, but how was this determined? Did you use a marker in the solution, or simply took the last chamber?

Answer: This comment relates to Comment SC1.3. Please refer to SC1.3 for a detailed answer.

Changes: See Comment SC1.3.

### **Comment RC1.5**

A comparison with the original, naturally-grown calcite would also be interesting.

Answer: A valuable suggestion for future research, but for the scope of this pilot study we consider the current data set sufficient.

Changes: -

### **Comment RC1.6**

What were the concentrations of these elements in the culture solutions; similar to sea water?

Answer: We did not determine the concentrations of Cl and F in the culture media directly. Since Cl and F are conservative elements following salinity, the concentrations are expected to resemble those in sea water with the same salinity.

Changes: The text has been modified to explicitly mention this: “The concentrations of Cl and F in the culture media were not directly determined. However, since Cl and F are conservative elements following salinity, the concentrations are expected to resemble those in sea water with the same salinity.”

### **Comment RC1.7**

The saturation state of the angulatus and marginalis experiments is very high. Were there any indicators of inorganic precipitation of calcite, which could have biased the results?

Answer: During the experiments there were no visual indicators of inorganic precipitation of calcite. The obtained specimens did not show visual overgrowth under the SEM.

Changes: We have added the following: “During the culture experiments there were no visual indicators of inorganic precipitation of calcite. Moreover, inspection with SEM of the measured specimens showed no inorganic calcite overgrowth.”

## Referee Comments Anonymous Referee #2

Roepert et al present NanoSIMS results looking at the distribution of chlorine and fluorine in cultured benthic foraminifera; two rotaliid species where calcite test walls are constructed via calcification around a primary organic sheet (hyaline calcification), and two miliolid species where test walls are constructed from calcite needles within an organic matrix. The preliminary results show that the calcification pathway of benthic foraminifera determines the incorporation and distribution of Cl, F, P and other elements in their calcite shells. The paper is interesting and well written and a good fit to Biogeosciences. One thing that is missing from the text relates to what kind of proxy the authors think the various halogen elements versus calcium ratios would represent? I have a couple of minor comments that can easily be addressed with minor revisions:

Thank you for your constructive feedback: our detailed responses are given below.

### Comment RC2.0

One thing that is missing from the text relates to what kind of proxy the authors think the various halogen elements versus calcium ratios would represent?

Answer: This information is given in the introduction in lines 42-44.

### Comment RC2.1

The study takes advantage of benthic foraminifera cultured for different purposes, under different conditions (Figure 4). Were all the samples cultured in the same artificial/natural seawater, and were halogen concentrations monitored?

Answer: The specimen were not cultured in the same artificial/natural seawater. However, the range of salinities created in the culture media by modifications of the natural and artificial seawater based culture media were larger than expected differences between natural and artificial seawater at the same salinity. For the experiments with *A. lessonii* and *A. tepida*, the culture media were produced from natural seawater, while for the culture experiments of *S. marginalis* and *A. angulatus*, artificial seawater based culture media were used. As halogen concentrations in seawater are tightly linked with salinity, we chose to determine, for practical reasons, salinity in the culture media stocks rather than halogen concentrations. See also answer to comment RC1.6.

Changes: More detail on culturing is provided in the methods section in the revised version: for the detailed changes, see comment RC1.6.

### Comment RC2.2

Several of the environmental parameters were calculated from other relationship (salinity-alkalinity) rather than measured. How constant would these parameters have been during the culture experiments?

Answer: As it was not feasible to measure these parameters in the small Petri dishes the foraminifera were grown in, the parameters were measured once when preparing the seawater stock. During the experiment, these variables may have varied slightly due to evaporation when feeding/cleaning. However, the media were replaced with fresh stock seawaters with the fixed parameters twice a week. Furthermore, culture media were in equilibrium with atmospheric  $p\text{CO}_2$ . Changes in DIC due to calcification are expected to be negligible given the high ratio of culture media volume to foraminiferal calcite per Petri dish.

Changes: We have added more information on the culturing to the Methods section: "Due to the small culturing volumes (Petri dishes), the parameters of the media could not be monitored during the experiments. However, potential changes due to evaporation during feeding or cleaning of the cultures are expected to be negligible, because the culture media were renewed regularly (twice a week), when compared to the large differences between the treatments."

### Comment RC2.3

It would be good to see a discussion of error estimates relating to the parameters the halogen/Ca are being compared with.

Answer: see also comment RC2.2. The ranges of the environmental parameters to which the halogen/Ca ratios are compared to, are in the extremes of what is found in natural seawaters, e.g. total alkalinity ranged from 1350 – 4477  $\mu\text{mol/kg}$ . We therefore expected that slight variations in the culture media had little impact with respect to the large differences between the culture media. Changes: We have added more information on the culturing to the Methods section.

#### **Comment RC2.4**

Furthermore, a brief discussion about halogen/Ca errors/variability also seems appropriate.

Answer: see Comment RC1.2.

Changes: see Comment RC1.2.

#### **Comment RC2.5**

All results are grouped together in Figure 3 and 4. Why would you expect a similar relationship between halogen/Ca and environmental parameters in hyaline and miliolid species?

Answer: Since this is the first detailed study of Cl and F incorporation into benthic foraminiferal calcite, we presented the data both in terms of co-variance with cations that have been investigated in detail before and in terms of carbonate system parameters. We made no a priori assumption about the absence or presence of a relationship. When halogen incorporation would not be dominated by biomineralization pathway differences of rotaliid and miliolid species, but governed (mainly) by environmental parameters, then a relationship may be expected, as discussed in lines 199-200.

#### **Comment RC2.6**

Correlations. Tone down discussion concerning correlations as only very few specimens were used of the same species etc in abstract and results section.

Answer: This comment echoes RC1.1. We are aware that the number of specimens measured in this study does not allow a robust interpretation of the effects of environmental parameters. We have nevertheless included figures showing our data in comparison to culture media properties for visualization.

Changes: see changes to comment RC1.1.

#### **Comment RC2.7**

Spatial distribution of halogen/Ca (Figure 1). For the hyaline species higher values are found in the primary organic sheet for all three halogens. Have the authors taken into consideration that Ca in the primary organic sheet will be much lower than in the calcite? Halogen/Ca ratios are hence higher, but it doesn't mean that halogens are actually higher in concentration than they are in the calcite. Do the anion counts show elevated concentrations in these bands?

Answers: Yes, we have taken into consideration that Ca intensities may be lower in the locations of the organic sheet compared to the calcite. However, due to the spatial resolution this is hardly visible in the Ca intensities and the anion intensities are elevated in the locations of the bands, see added figure A6.

Changes: We have added Figure A6 to the appendix showing the elemental intensity profiles for the same transects as in Figure 1, illustrating that elevated halogen/Ca ratios at the locations of organic linings are not caused by lower Ca intensities. We furthermore added "These bands are not caused by lower Ca intensities at these locations (Figure A6)." to the Results section.

Other comments:

#### **Comment RC2.8**

Abstract: The discussion of the results is vague. What is meant by 'Cl and F were highly heterogeneous and correlated within the shell walls' (line 7, 8), and 'In these species Cl and P were correlated' (line 10)? was the correlation positive or negative, and how significant?

Answer: 'Cl and F were highly heterogeneous and correlated within the shell walls' (line 7, 8) refers to the spatial distribution of Cl and F within shell walls. 'In these species Cl and P were correlated' (line 10) refers to a positive spatial correlation of Cl and P.

Changes: the text was adapted as follows: "Cl and F were distributed highly heterogeneously within the shell walls, forming bands that were co-located with the bands observed in the distribution of phosphorus (significant positive correlation of both Cl and F with P;  $p < 0.001$ )" and "In these species Cl and P were spatially positively correlated ( $p < 0.001$ )"

#### **Comment RC2.9**

Lines 14, 15 'We further propose that in the miliolid species Cl may be incorporated as a solid solution of chlorapatite or associated with organic molecules in the calcite'. It is unclear what is meant with solid solution? Do you mean chloroapatite that has dissolved?

Answer: The term "solid solution" is a standard term used in thermodynamics and mineralogy for mixtures of solid phases that have similar crystal structures (similar to aqueous solutions in aquatic environments).

#### **Comment RC2.10**

Perhaps not use the word organic lining as a pseudonym for primary organic sheet, as foraminifera sometimes have an organic lining on the inside of the test.

Answer: We acknowledge that foraminifera can have organic linings additional to the primary organic sheet.

Changes: We added "Here, we use the term organic linings to refer collectively to the primary organic sheet and other organic linings in the shell wall." to the discussion for clarity.

#### **Comment RC2.11**

4.1 How could you check if fluorite or fluorapatite are the incorporation mechanism for fluorine in calcite? Has there been a discussion about this with regards to aragonite which is also higher in F?

Answer: Spectroscopic techniques such as synchrotron could potentially identify incorporation mode of F in foraminiferal calcite, hence, whether fluorite or fluorapatite may play a role here. This requires further investigations outside the scope of this study. The incorporation mechanism for F into aragonite can be attributed to ion exchange with carbonate ion (Ichikuni, Chemical Geology 1979) and to the best of our knowledge alternatives have not been discussed yet.

Changes: We have added text to the discussion to point towards future research options.

#### **Comment RC2.12**

Figure 5 What is new here compared with previous work? Needs appropriate referencing.

Answer: done.

Changes: References were added to the figure caption.

#### **Comment RC2.13**

Figure A1 SEM images are mirrored. Please change back!

Answer: The SEM images in Figure A1 are mirrored on purpose to represent the orientation of the nanoSIMS images. Mirroring them back would complicate visual comparison of the SEM images and nanoSIMS images. We therefore decided to leave the SEM images in their mirrored state.

Changes: we have flipped back the scale bar annotation for better readability and added "SEM images are flipped horizontally to facilitate navigation in the NanoSIMS instrument, where the secondary ion images are horizontally mirrored." to the caption of Figure A1.



# Distribution of chlorine and fluorine in benthic foraminifera

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**Abstract.** Over the last decades a suite of inorganic proxies based on foraminiferal calcite have been developed, of which some are now widely used for paleoenvironmental reconstructions. Studies of foraminiferal shell chemistry have largely focused on cations and oxyanions, while much less is known about the incorporation of anions. The halogens fluoride and chloride are conservative in the ocean, which makes them candidates for reconstructing paleoceanographic parameters. However, their potential as a paleoproxy has hardly been explored, and fundamental insight in their incorporation is required. Here we used nano-scale secondary ion mass spectrometry (NanoSIMS) to investigate, for the first time, the distribution of Cl and F within shell walls of four benthic species of foraminifera. In the rotaliid species *Ammonia tepida* and *Amphistegina lessonii* Cl and F were ~~highly heterogeneous and correlated~~ distributed highly heterogeneously within the shell walls, forming bands that were co-located with the ~~banded bands observed in the~~ distribution of phosphorus (significant positive correlation of both Cl and F with P;  $p < 0.01$ ). In the miliolid species *Sorites marginalis* and *Archaias angulatus* the distribution of Cl and F was much more homogeneous without discernible bands. In these species Cl and P were ~~correlated~~ spatially positively correlated ( $p < 0.01$ ), whereas no correlation was observed between Cl and F or between F and P. Additionally, their F content was about an order of magnitude higher than in the rotaliid species. The high variance in the Cl and F content in the studied foraminifera specimens could not be attributed to environmental parameters. Based on these findings we suggest that in the rotaliid species Cl and F are predominately associated with organic linings. We further propose that in the miliolid species Cl may be incorporated as a solid solution of chlorapatite, or associated with organic molecules in the calcite. The high F content together with the lack of correlation between Cl and F or P in the miliolid foraminifera suggests a fundamentally different incorporation mechanism. Overall, our data clearly show that the calcification pathway employed by the studied foraminifera governs the incorporation and distribution of Cl, F, P and other elements in their calcite shells.

## 20 1 Introduction

Foraminifera are widely used to reconstruct paleoenvironments and climates based on relative species abundances and the chemical or isotopic composition of their shells. Apart from the carbon and oxygen isotopic composition, most inorganic proxies based on foraminiferal calcite involve cations or their isotopes (Boyle, 1981; Elderfield et al., 1996; Lea et al., 1999;

Allen et al., 2016), which can substitute for calcium in the calcite lattice. While there are also proxies based on the incorporation  
25 of oxyanions such as iodate, sulphate and borate, and their isotopes (Lu et al., 2010; Paris et al., 2014; Yu et al., 2007; Rae  
et al., 2011), much less is known about the incorporation of anions such as the halogens Cl, F and Br that are less likely to  
substitute for the carbonate ion (Kitano et al., 1975; Okumura et al., 1983, 1986).

The halogen anions are conservative in the ocean, which makes them potential candidates for reconstructing paleoceanographic  
parameters. Chlorine as a major constituent of sea salt is the most abundant halogen in seawater, followed by bromine,  
30 while fluorine concentrations are in the ppm range (e.g., Kendrick, 2018, and references therein). However, even though  $\text{Cl}^-$  is  
the most abundant (an)ion in seawater, the Cl content of inorganic and biogenic calcites is low as the  $\text{Cl}^-$  anion does not fit well  
into the calcite lattice (Tokuyama et al., 1972; Kitano et al., 1975). In contrast, the  $\text{F}^-$  anion has a much higher compatibility  
in many minerals compared to the heavier halogens, which can be attributed to its much smaller ionic radius (Kendrick, 2018).  
While seawater is enriched with the halogens chlorine and bromine with respect to the primary mantle (Kendrick, 2018),  
35 fluorine is strongly depleted in seawater with calcium carbonates being the major sink of dissolved fluoride in the oceans  
(Carpenter, 1969).

Marine carbonates have F/Ca ratios ranging between 0.1–3.5  $\text{mmol mol}^{-1}$  and 4.0–6.5  $\text{mmol mol}^{-1}$  in calcite and aragonite,  
respectively (Carpenter, 1969), while Cl/Ca ratios range between 9–18  $\mu\text{mol mol}^{-1}$  and 53–71  $\mu\text{mol mol}^{-1}$  in inorganically  
precipitated calcite and aragonite, respectively (Kitano et al., 1975). The fluorine content of planktic foraminifera co-varies  
40 not only with  $\delta^{18}\text{O}$  (Rosenthal and Boyle, 1993; Opdyke et al., 1993; Rosenthal et al., 1997), but also with Mg and Sr content  
(Opdyke et al., 1993), and varies mostly in response to species-specific biological factors (Rosenthal and Boyle, 1993; Opdyke  
et al., 1993; Rosenthal et al., 1997). Furthermore, foraminiferal F content appears to be highly susceptible to diagenesis. Indeed,  
the release of F to porewater has been shown to be a proxy for carbonate dissolution during early diagenesis (Rude and Aller,  
1991). The chlorine content of biogenic calcite has been proposed as a potential direct salinity proxy (Wit et al., 2013), but  
45 there are very few data on Cl in foraminifera (Szafranek and Erez, 1993; Erez, 2003).

Not much is known about how these anions are incorporated into foraminiferal shells. Possible incorporation modes include  
lattice-bound, interstitial, solid solution (e.g. fluorite, apatite), or bound to organic templates within the foraminiferal shell  
walls. Possible environmental factors playing a role during the incorporation processes of Cl and F may be related to pH home-  
ostasis in the calcification fluid as  $\text{Cl}^-$  and  $\text{F}^-$  are the dissociated compounds of a strong and weak acid, respectively. Tanaka  
50 et al. (2013) showed that fluorine incorporation in non-symbiotic corals was governed by the carbonate ion concentration in  
solution because of ion-exchange between fluoride and carbonate. A similar link has been suggested between foraminiferal  
fluorine content and carbonate ion concentration (Rosenthal and Boyle, 1993; Opdyke et al., 1993) as well as the presence of  
symbionts (Opdyke et al., 1993).

Furthermore, the spatial distribution of incorporated elements within foraminiferal shells can shed light on the incorporation  
55 mechanism. To the best of our knowledge, the spatial distribution of F has not been investigated yet, while Cl was previously  
found at the location of organic linings in shell walls of *A. lobifera* (Szafranek and Erez, 1993; Erez, 2003). Recently, also  
the iodine micro-distribution in shell walls of *U. striata* was found to spatially correlate with organics (Glock et al., 2019).  
This suggests that halogen incorporation may be regulated by foraminiferal biomineralization pathways, although biogenic

carbonate formation is ultimately expected to be governed by the same controlling factors as inorganic carbonate formation.

60 The biomineralization pathway has consequences for trace element incorporation, as reflected in the occurrence of bands of higher and lower concentrations of cations (e.g. Mg, Sr, Ba, Na) in perforate hyaline foraminiferal shell walls (Eggins et al., 2004; Sadekov et al., 2005; Kunioka et al., 2006; Branson et al., 2015; Jonkers et al., 2016; Geerken et al., 2018, 2019). This banding has been attributed to chamber addition in perforate hyaline foraminifera: an additional high-concentration band appears each time a new calcite lamella is added to the shell, which also covers previous chambers (Nehrke et al., 2013).

65 Similarly, spine and the spine attachment zone of planktic foraminifera are enriched in Na while depleted in Mg, and vice versa, respectively (Branson et al., 2016; Mezger et al., 2019; Bonnin et al., 2019).

Here we investigated, for the first time, the Cl and F distribution within foraminiferal shell walls of four benthic species on a sub-micron scale with NanoSIMS. Two rotaliid (hyaline) benthic species (*Ammonia tepida* and *Amphistegina lessonii*) and two miliolid (porcellaneous) benthic species (*Sorites marginalis* and *Archaias angulatus*) were investigated to cover benthic

70 foraminifera with fundamentally different biomineralization pathways. Furthermore, one species is symbiont-barren (*Ammonia tepida*), whereas the other three species are bearing photosynthetic symbionts.

## 2 Materials and Methods

### 2.1 Specimen selection and basic characterization

Six specimens of four species (two rotaliid and two miliolid species, respectively) from culturing experiments were investigated

75 in this study (Table 1). The use of laboratory-grown specimens has the advantage that their growth conditions were controlled and that potential effects of diagenesis on their shell halogen chemistry can be excluded. The *A. angulatus* and *S. marginalis* specimens were collected in Sint Eustatius (Oranjestad Bay, 17.479751°N –62.987273°W). The culture experiments with *A. angulatus* and *S. marginalis* were conducted in the same manner as described in van Dijk et al. (2017), with the exception of media preparation. Culture media of different salinities were prepared by mixing natural 0.2 µm filtered seawater with

80 deionized water and 'instant ocean' salt, to obtain a range in salinities between 25–45. Calcein was added during the course of the experiment, and fluorescence images were used to identify newly precipitated calcite. The *A. lessonii* specimens are from Burger's Zoo, NL (van Dijk et al., 2019), with the culture conditions being reported in van Dijk et al. (2019). The specimens of *A. tepida* were collected on a tidal flat near Den Oever, the Wadden Sea, NL (Hayward et al. 2004), with the culture conditions being described in Geerken et al. (2018). For both the cultures of *A. lessonii* and *A. tepida*, 2-3 chambered juveniles were

85 transferred into Petri dishes containing culture media with adjusted salinity and alkalinity, where the specimens precipitated additional chambers. Prior to embedding, all specimens were cleaned using an adapted Barker protocol (Barker et al., 2003), only applying the organic removal/oxidation step, in which NaOH was replaced by NH<sub>4</sub>OH, as described in detail in Geerken et al. (2018). During the culture experiments there were no visual indicators of inorganic precipitation of calcite. Moreover, inspection with SEM of the measured specimens showed no inorganic calcite overgrowth.

90 Parameters of the carbonate system in the culture media stock, including alkalinity, carbonate ion concentration and saturation state with respect to calcite, were calculated from the measured DIC concentration, pH, salinity and temperature (Table 1).

This was done in R (R Core Team, 2018) using the package *seacarb* (Gattuso et al., 2018). The equilibrium constants for the carbonate system  $K_1$  and  $K_2$  were taken from Lueker et al. (2000). Because DIC was not measured in the culture of *A. lessonii*, we first applied the salinity-alkalinity relationship for the North Atlantic (Lee et al., 2006) to estimate the culture medium alkalinity, based on which we then calculated the remaining carbonate system parameters. The concentrations of Cl and F in the culture media were not directly determined. However, since Cl and F are conservative elements following salinity, the concentrations are expected to resemble those in sea water with the same salinity. Due to the small culturing volumes (Petri dishes), the parameters of the media could not be monitored during the experiments. However, potential changes due to evaporation during feeding or cleaning of the cultures are expected to be negligible, because culture media were regularly renewed (twice a week), when compared to the large differences between the treatments.

Prior to sample preparation for NanoSIMS analyses, each specimen was characterized with respect to its Na, Mg, Sr and Ba content. This was done by LA-ICP-MS for *A. tepida* and *A. lessonii* as previously described (Geerken et al., 2018; van Dijk et al., 2019). For *A. angulatus* and *S. marginalis* LA-ICP-MS analyses were performed using the same methodology as described in Geerken et al. (2018). For each specimen El/Ca ratios were determined on two or more chambers and averaged (Table A1).

## 2.2 Sample preparation and SEM imaging

The selected specimens of *A. tepida*, *A. lessonii*, *S. marginalis* and *A. angulatus* were embedded in epoxy resin (Araldite 2020) under vacuum and subsequently polished to expose cross-sections perpendicular to the shell walls (see Geerken et al. (2019) for details). Although epoxy resins contain high amounts of chlorine, we are confident that the halogens measured in the foraminiferal calcite are not due to contamination with resin nor an artifact of redeposition during the sputtering process (Roepert, 2019). The first polishing steps used wet grinding papers of decreasing coarseness (HERMES, WS Flex 18C, 230 mm, P 800 and 219 ATM, SiC wet grinding paper, grain 4000) followed by agglomerated alpha alumina powder (Struers AP-A powder, grain size 0.3  $\mu\text{m}$ ) and SiO<sub>2</sub> powder (Logitech SF1 Polishing Suspension, grain size 0.035  $\mu\text{m}$ ). The polished samples were sputter-coated with 20 nm of Au using a sputter coater (JEOL JFC-2300HR high resolution fine coater with JEOL FC-TM20 thickness controller), after which they were imaged using a JEOL Neoscope II JCM-6000 table-top SEM to identify suitable areas for NanoSIMS analysis (Figure A1).

## 2.3 NanoSIMS imaging

The fields of view for NanoSIMS imaging were carefully selected using SEM images on the basis of the position in the specimen and the quality of the surface preparation. Where possible, distal chambers were measured, but more proximal chambers were preferred if their cross-sectional surfaces appeared flatter or cleaner. NanoSIMS analysis was performed with the Cameca NanoSIMS 50L instrument available at Utrecht University. Using an element standard (SPI Supplies, 02757-AB 59 Metals & Minerals Standard), magnetic field and exact positions of the electron multiplier detectors were adjusted to enable detection of negative secondary ions  $^{12}\text{C}^-$ ,  $^{16}\text{O}^-$ ,  $^{19}\text{F}^-$ ,  $^{31}\text{P}^-$ ,  $^{35}\text{Cl}^-$ ,  $^{37}\text{Cl}^-$ , and  $^{40}\text{Ca}^{16}\text{O}^-$ . The secondary ions were sputtered from the sample surface using an 8 kV primary Cs<sup>+</sup> ion source.

125 Because the primary ion beam was positive, calcium had to be detected as  $^{40}\text{Ca}^{16}\text{O}^-$  and not as  $^{40}\text{Ca}^+$ . However, because the  
Ca:O stoichiometry in calcite with trace amounts of organics is fixed, we assumed that the measured distribution of  $^{40}\text{Ca}^{16}\text{O}$   
well approximated the distribution of Ca. This assumption was supported by the good correlation between the secondary ions  
 $^{40}\text{Ca}^{16}\text{O}^-$  and  $^{16}\text{O}^-$  detected from the calcite (Figure A2).  $^{31}\text{P}$  was measured as a tracer for organics in the calcite (Geerken  
et al., 2019), whereas  $^{12}\text{C}$  was measured to help distinguish between resin and calcite. To ensure that the detection of  $^{35}\text{Cl}$   
130 was not influenced by possible isobaric interferences such as  $^{16}\text{O}^{18}\text{O}^1\text{H}$  and  $^{34}\text{S}^1\text{H}$ , we used sufficiently high mass resolution  
power ( $\text{MRP} > 5113$ ) and additionally measured the isotope  $^{37}\text{Cl}$  as well. The obtained  $^{37}\text{Cl}/^{35}\text{Cl}$  ratio differed from the natural  
abundance ratio of 0.320 by no more than 0.015, confirming that the influence of isobaric interferences for the detection of  
Cl was negligible. Similarly, separation of  $^{19}\text{F}$  from the possible interference by  $^{18}\text{O}^1\text{H}$  was achieved by using  $\text{MRP} > 2214$ ,  
whereas interferences from molecules such as  $^{12}\text{C}^7\text{Li}$ ,  $^{13}\text{C}^6\text{Li}$  or  $^{16}\text{O}^1\text{H}_3$  are highly unlikely.

135 Before each analysis the area of interest was pre-sputtered for 10–15 min until secondary ion counts stabilized. Subsequently,  
ion count images were acquired by rastering the primary beam (dwell time of  $1\text{ ms pixel}^{-1}$ ) over the sample surface using the  
diaphragm and slit settings listed in Table A2. The primary beam current on the sample surface ranged between 0.5–2 pA  
depending on the size of the imaged area. The spatial resolution ranged between 50–100  $\text{nm pixel}^{-1}$ . All analyses employed  
an e-gun to avoid charging of the sample surface. Because some of the secondary ion counts were very low, the imaged areas  
140 were measured multiple times (250–1000, depending on the sample) and the signals from the individual planes were aligned  
and accumulated.

## 2.4 Data processing

NanoSIMS data were processed with the freeware program Look@NanoSIMS (Polerecky et al., 2012). After alignment and  
accumulation of the image data, regions of interest (ROIs) corresponding to foraminiferal calcite were drawn by hand based  
145 on the  $^{40}\text{Ca}^{16}\text{O}^-$  image. With the additional use of the  $^{12}\text{C}^-$  and  $^{35}\text{Cl}^-$  images, areas of exposed resin or pores within the  
foraminiferal walls were identified and excluded from the final analysis.

Due to the lack of reliable calibration standards, and because Ca was measured as the molecular ion  $^{40}\text{Ca}^{16}\text{O}^-$ , the EI/Ca  
ratios are reported in this study by the ratios between the raw data, i.e., the secondary ion counts  $\text{EI}^-$  and  $^{40}\text{Ca}^{16}\text{O}^-$ . Although  
not fully quantitative, the ratios calculated in this way are comparable between the different foraminifera specimens and species  
150 because the secondary ion counts detected by NanoSIMS are linearly proportional to the concentration of the corresponding  
element, and because all the measurements were done in a similar biogenic calcite matrix using the same pre-sputtering and  
measuring protocol.

To ensure that the EI/Ca ratios were not affected by insufficient pre-sputtering, depth profiles of the  $\text{EI}^- / ^{40}\text{Ca}^{16}\text{O}^-$  ion count  
ratios were inspected for each ROI, and the planes where the ratios showed a significant trend with depth were excluded from  
155 the final analysis. Lateral profiles of the  $\text{EI}^- / ^{40}\text{Ca}^{16}\text{O}^-$  ion count ratios perpendicular to the shell surface were extracted from  
the accumulated NanoSIMS images. The width of the profile line, which corresponds to the amount of averaged lateral profiles,  
was set to 20 pixel to increase the signal-to-noise-ratio. [As such, lateral profiles that cover a representative fraction of a shell  
wall may be comparable to LA-ICP-MS profiles, albeit with a higher resolution.](#)

To investigate the spatial correlation of  $\text{El}^- / ^{40}\text{Ca}^{16}\text{O}^-$  ion count ratios, ROIs were drawn on the NanoSIMS images in  
160 Look@NanoSIMS in such a way, that regions of higher and lower ion count ratios on the foraminifera were separated into  
different ROIs to separate the spatial variability. That way, 40 to 47 separate data points (ROIs) per species, grouped per  
specimen, were extracted from the NanoSIMS images. Subsequently, correlation matrices were calculated for the accumulated  
ion count ratios in those ROIs using the `corrplot` package (Wei and Simko, 2017) in R (R Core Team, 2018).

### 3 Results

#### 165 3.1 Spatial distribution of chlorine and fluorine

The halogens Cl and F show distinct banding in the rotaliids, in particular in *A. tepida* (Figure 1). [These bands are not caused by  
170 lower Ca intensities at these locations \(Figure A6\)](#). Moreover, in the rotaliids, maxima of Cl/Ca and F/Ca are co-located with  
those of P/Ca, and correlate well in *A. tepida*, while the correspondence between P/Ca and F/Ca is moderate in *A. lessonii*, and  
spatially rather complex for Cl/Ca (see lateral profiles in Figure 1). Furthermore, the contrast between the high-intensity and  
low-intensity bands in F/Ca, Cl/Ca and P/Ca is higher in *A. tepida* than in *A. lessonii*. In the miliolid foraminifers no banding  
of neither halogens nor P is visible, with the exception of a slight elevation in Cl and P in areas of an image that were identified  
as a suture in SEM images. Lateral profiles in *A. angulatus* show a correlation of Cl with P, and no correlation of F with Cl or  
P. The lateral profile through the shell wall of *S. marginalis* shows similar patterns as the one of *A. angulatus*. The F/Ca ion  
count ratios in the miliolids *A. angulatus* and *S. marginalis* are in the same range and one order of magnitude higher than those  
175 in rotaliid *A. tepida* and *A. lessonii* (Figures 1 and 2). The Cl/Ca and P/Ca ion count ratios are in the same order of magnitude  
in all four species (Figures 1 and 2).

Ion count ratios of F/Ca and Cl/Ca correlate with each other in *A. tepida*, while there is no correlation in *A. lessonii* and the  
miliolids (Figure 2A and A3). Both F/Ca and Cl/Ca are correlated with P/Ca in the rotaliids, while only Cl/Ca is correlated with  
P/Ca in the miliolids (Figure 2B,C). All correlations described here are significant to a level of  $p < 0.001$ ;  $R^2$  and p-values of  
180 the correlations are reported in Figure A3. These correlations are also seen when the elements F, Cl and P are normalized to O  
instead being normalized to Ca (Figure A5).

#### 3.2 Relation with cation incorporation and culture media properties

In all four species, the Cl content does not correlate to any of the elemental ratios measured by LA-ICP-MS (upper panels in  
Figure 3). However, the elevated F/Ca ratios in the miliolid foraminifera coincide with elevated Mg/Ca and Ba/Ca, which also  
185 are an order of magnitude higher in these species than in the rotaliid foraminifera (lower panels in Figure 3). Our data show no  
correlation of Cl/Ca or F/Ca with neither Na/Ca nor Sr/Ca (Figure 3).

Our data show no correlation of Cl/Ca or F/Ca with salinity or temperature (Figure A4). Furthermore, crossplots of the  
NanoSIMS Cl/Ca and F/Ca ion count ratios show no correlation with carbonate system parameters for Cl/Ca (Figure 4). How-

ever, NanoSIMS F/Ca ion count ratios are higher in the miliolid foraminifera, which were cultured at higher DIC, corresponding  
190 to higher alkalinity and carbonate ion concentration as well (Figure 4).

## 4 Discussion

### 4.1 Limited environmental control on Cl and F incorporation into foraminiferal shells

The NanoSIMS data presented clearly show that biomineralization pathways govern the incorporation and distribution of Cl  
195 and F within foraminiferal shells: the rotaliid species show distinct banding in Cl, F and P, while the F-rich miliolid species  
do not. Biologic control is known to affect incorporation of most elements into foraminiferal shells, while at the same time  
relationships with environmental conditions have proven robust tools for paleo reconstructions (Eggins et al., 2004; Kunioka  
et al., 2006; Paris et al., 2014; Spero et al., 2015; Fehrenbacher et al., 2017; Geerken et al., 2019). In our data set comprised of  
a very limited amount of specimens, we see no overall trend in Cl/Ca ratios in foraminiferal calcite with chemical properties of  
200 the culture media. The high intra-shell variability in rotaliid foraminifera and the spatial correlation with P on the location of  
organic linings suggest that Cl is associated with organic linings in rotaliid foraminiferal shells. Furthermore, Cl/Ca is highly  
variable within a single section through a foraminiferal wall in all the species measured and the range of Cl/Ca ratios is similar  
in all investigated specimens.

The overall absence of trends with environmental conditions as well as the high intra-specimen variability lowers the confi-  
205 dence in potentially using Cl/Ca for paleo reconstructions. However, as this study does not cover a range of physicochemical  
parameters for a single species, but rather presents a collection of a limited number of specimens of different species that  
were also grown in different conditions, we cannot exclude that any of the presented physicochemical parameters may exert  
an influence on the Cl content of foraminifera on a species-specific level. ~~A~~ We emphasize that a definite conclusion regarding  
proxy applicability would require culturing studies including 20–30 specimens per species per environmental condition.

210 Moreover, there is no discernable trend of Cl/Ca ratios in foraminiferal calcite with any of the measured trace elements  
(Figure 3). Cl/Ca ratios are in the same range for species with low-Mg calcite (rotaliid) as they are for those with high-Mg  
calcite (miliolid), suggesting that chlorine incorporation is systematically different from the incorporation of these cations.  
In inorganically precipitated calcite, chlorine contents are an order of magnitude lower than sodium contents (Kitano et al.,  
1975), suggesting that Cl is incorporated neither as fluid inclusions (Wit et al., 2013) nor as solid solutions of NaCl into calcite.  
215 As chlorine content seems not to reflect any environmental parameter, and Cl/Ca correlates well with P/Ca in all the species  
investigated here, we suspect that chlorine incorporation into foraminiferal calcite is closely related to organic molecules  
involved in calcification ~~or to~~. Another possibility may be a solid-solution between calcite and a mineral containing both Cl  
and P, such as chlorapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH},\text{Cl})$ ). As NanoSIMS cannot distinguish between these options, further research  
involving spectroscopic techniques is required to elucidate the nature of Cl incorporation in calcite.

220 The F/Ca ratios in the miliolid species are about an order of magnitude higher than those in the rotaliid species. The elevated  
F/Ca ratios in miliolids coincide with overall higher  $\text{CO}_3^{2-}$ -ion concentration in the culture media of the miliolid species.

This might indicate a relation between foraminiferal F/Ca ratios and carbonate ions, but the relationship is inverse to what one would expect for inorganic ion exchange (Ichikuni, 1979) and what has been observed in corals (Tanaka et al., 2013). However, the high intra-shell variability in F/Ca ratios of single specimens and the co-variation of environmental conditions with mineralization pathway complicates attributing F/Ca ratios to environmental parameters. Species-specific culturing studies could provide more insight into whether F/Ca ratios of benthic foraminifera on a species-specific level correlate with environmental conditions, as then the effect of different biomineralization pathways would not hamper interpretation as is the case in our data set.

Opdyke et al. (1993) suggested that the presence of photosynthetic symbionts in foraminifera impacts their F/Ca ratio. Symbionts influence the intracellular carbonate chemistry by photosynthesis, which could link to fluoride incorporation via the intracellular  $\text{CO}_3^{2-}$  ion activity. *A. tepida* is the only symbiont-barren species we investigated, and indeed its F/Ca ratios are lower than in the miliolid symbiont-bearing species. However, the symbiont-bearing rotaliid *A. lessonii* exhibits the lowest mean F/Ca ratios, which are in the same order of magnitude as in *A. tepida*, but less variable throughout the shell wall. We therefore conclude that F/Ca ratio is unlikely directly related to the presence of symbionts in foraminifera.

Notably, F/Ca ratios are higher in specimens with a higher Mg and Ba content (Figure 3). A correlation of trace element content with Mg content within and between species has been found for several elements, including Sr, Zn, and Na (Evans et al., 2015; van Dijk et al., 2017; Geerken et al., 2018), and also F (Opdyke et al., 1993). The miliolid species have generally a much higher Mg content than the rotaliid species and their biomineralization mechanisms are thought to be substantially different (Figure 5). The fact that higher F content corresponds with higher Mg content may point towards a strong biological control on F incorporation. Fluorine may be incorporated in solid solutions. Fluorite ( $\text{CaF}_2$ ) solid solution has been suggested as the incorporation mechanism for fluorine in calcite (Carpenter, 1969), but also fluorapatite ( $\text{Ca}_5(\text{PO}_4)_3(\text{OH},\text{F})$ ) solid solution would be a possible option. [Using spectroscopic techniques such as synchrotron could potentially identify the incorporation mode of F in foraminiferal calcite, hence, whether fluorite or fluorapatite may play a role. This requires further investigations outside the scope of this study.](#)

#### 4.2 Cl and F incorporation in foraminifera is primarily controlled by biomineralisation pathway

In the two species of rotaliid foraminifera that were investigated here, Cl and F show strong banding. The Cl and F bands are co-located with P in the foraminiferal shell walls. Since phosphorus is present in organic molecules like phospholipids in membranes, P/Ca can be used to image organic linings in between the lamella in hyaline foraminiferal shell walls, as demonstrated in Geerken et al. (2019). [Here, we use the term organic linings to refer collectively to the primary organic sheet and other organic linings in the shell wall.](#) In *A. tepida*, the correlation of Cl and F with P is tight, and we conclude that both elements are primarily associated with the organic linings. In *A. lessonii*, the peaks in Cl/Ca and F/Ca lateral profiles are also co-located with peaks in P/Ca. However, in the specimens we analyzed, there seems to be substantial additional Cl and F also in some calcite lamella, as can be seen in the lateral profiles. Moreover, the contrast between high-intensity bands and low-intensity bands is less prominent in *A. lessonii* than in *A. tepida*. We suggest that also in *A. lessonii*, association with organic linings is the primary mode of incorporation of both elements in the foraminiferal shells. Using NanoSIMS and the very same



species and specimens, Geerken et al. (2019) reported co-occurrence of organic linings and banding of Mg, Na, Sr, K, S, P and N. Moreover, they showed that elemental incorporation in *A. lessonii* was overall higher than in *A. tepida*, consistent with our observations for the halogens (Figures 1, 2).

In the miliolids, the distributions of Cl and F are distinctly different from those of the rotaliids: since miliolids do not calcify by adding subsequent lamella of calcite (Figure 5), no patterns of alternating high and low concentration banding are visible. Moreover, Cl and F are spatially not correlated throughout the shell walls of these miliolids, indicating that Cl and F may have different modes of incorporation. The correlation of Cl and P within the shell wall supports the hypothesis that Cl is incorporated in the calcite as a solid solution of chlorapatite, a mineral containing both Cl and P, or associated with organic molecules as for the rotaliids, but then distributed in a less organized way (no banding) within the calcite. The type of organics being present in foraminiferal calcite is determined by the precipitation pathway: rotaliids initiate calcification around a primary organic sheet (POS) and cover their shell with organic linings, while miliolid shells comprise of randomly oriented calcite needles held together in an organic matrix. As these organics may differ in their P content, it is possible to measure comparable P contents in both rotaliid and miliolid calcite, even though their absolute organic matter content within the calcite is expected to be different. Similar Cl content in both rotaliids and miliolids may thus be due to differences in the composition of the organics or may hint to an incorporation via a different pathway. Alternatively, apatite may form via the adsorption of phosphate to calcite and amorphous calcium phosphates at low Mg concentrations in solution (Martens and Harriss, 1970; Millero et al., 2001). The incorporation of Cl via chlorapatite could also explain the spatial correlation of Cl and P in the species we analysed. If F would be incorporated similarly as Cl, we would expect the F and Cl distribution to be comparable. As this is not the case, we conclude that F is incorporated primarily in a different way than Cl, e.g. as a solid solution of fluorite, as suggested before (Rosenthal and Boyle, 1993).

The observed difference between F-rich miliolids lacking organic linings and clear banding of trace elements on the one hand, and the rotaliids with F, Cl and P rich bands on the other hand, is consistent with known differences in calcification mechanisms (Figure 5) that have developed during the evolution of foraminifera (Debenay et al., 1996; Bentov and Erez, 2006). Hyaline (including the rotaliid) foraminifera precipitate calcite onto organic templates within an extracellular but confined space, and add a new lamella to the entire shell each time they produce a new chamber (Hemleben et al., 1986; de Nooijer et al., 2014). For some intermediate Mg-calcite producing foraminifera (like *A. lobifera* or also *A. lessonii*), transport of vesicles to the site of calcification has been observed suggesting controlled biomineralization (at least partly) from internal Ca and carbonate pools (e.g., de Nooijer et al., 2009). Furthermore, in hyaline foraminifera, selective ion transport to the site of calcification via trans-membrane pumping of elements is discussed as the biological control on biomineralization (Nehrke et al., 2013; de Nooijer et al., 2014; Toyofuku et al., 2017). In contrast, porcelaneous miliolid foraminifera produce a wall of high-Mg calcite with thin inner and outer layers connected by a thick middle layer of crystal needles (Hemleben et al., 1986; Debenay et al., 1996), which are glued together by an organic matrix. Miliolid foraminifera are generally thought to precipitate calcium crystals in intracellular vesicles prior to arranging them in the shape of the new chamber wall (Angell, 1980; Hemleben et al., 1986; Debenay et al., 1998, 2000; Bentov and Erez, 2006). Furthermore, there are species within the

290 suborder of the miliolids that show features of both biomineralization pathways, such as *Archaias angulatus*, which appears to precipitate calcite at the site of the new chamber wall, opposed to other miliolid species (Wetmore, 1999).

During calcification, miliolids enclose seawater vesicles (Figure 5) and then produce calcite rich in various cations (van Dijk et al. (2017), Table A1) and F (Figure 1). The rotaliids such as *A. tepida* may use highly selective ion channels and organic layers to deposit new calcite. As a consequence, these calcites are low in Mg, Ba and the halogens (F, Cl) and show distinct  
295 banding. The rotaliid *A. lessonii* produces calcite with intermediate Mg contents and less distinct banding for cations (Geerken et al., 2019), indicative of less biological control on ion transport and calcite deposition than in *A. tepida*. Our results for P, Cl and F support this.

## 5 Conclusions

Here we investigated for the first time the spatial distribution of the halogens Cl and F in foraminiferal shell walls. In the  
300 rotaliid benthic species *Ammonia tepida* and *Amphistegina lessonii*, Cl and F are distributed in bands within the chamber walls, which co-locate with P banding. In the miliolid benthic species *Sorites marginalis* and *Archaias angulatus* Cl and F were not found to occur in bands. However, the rather homogeneously-distributed Cl was found to correlate with P content, while F did not correlate with either P or Cl. Based on these findings we suggest that Cl and F are predominately associated with organic linings in the rotaliid species. We further propose that Cl may be incorporated in miliolid species as a solid solution of  
305 chlorapatite or be associated with organics. Our data in the miliolid species suggests that F is incorporated in a different way than Cl, as F does not correlate with P. [Further research is required to evaluate the potential of Cl and F in shells of benthic foraminifera for proxy applications.](#)

*Data availability.* The data presented in this study are available at doi:10.4121/uuid:9951e801-5574-498e-b375-fa6941a0f071.

*Author contributions.* JJM, GJR and AR designed the experiments. EG prepared the samples for NanoSIMS analyses. EG and AR conducted  
310 the SEM analyses. AR conducted the NanoSIMS analyses. AR and LP performed the data analysis. AR interpreted the data, prepared the figures and wrote the manuscript text with contributions from all authors.

*Competing interests.* The authors declare no competing interests.

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**Table 1.** Studied foraminifera specimens and the corresponding culture conditions.

#	species <sup>1</sup>	salinity	T [°C]	DIC [μmol/kg]	alkalinity [μmol/kg]	pH	CO <sub>3</sub> <sup>2-</sup> [μmol/kg]	Ω <sub>calcite</sub>
1 <sup>†</sup>	<i>A. tepida</i> (R)	25.2	25	1087	1350	8.32	162	4.22
2 <sup>‡</sup>	<i>A. lessonii</i> (R)	35.2	21.2	2069	2314	8.00	177	4.24
3*	<i>A. angulatus</i> (M)	40	25	3861	4477	8.10	506	11.67
4*	<i>A. angulatus</i> (M)	30	25	2644	3153	8.27	399	10.00
5*	<i>S. marginalis</i> (M)	30	25	2644	3153	8.27	399	10.00
6*	<i>S. marginalis</i> (M)	40	25	3861	4477	8.10	506	11.67

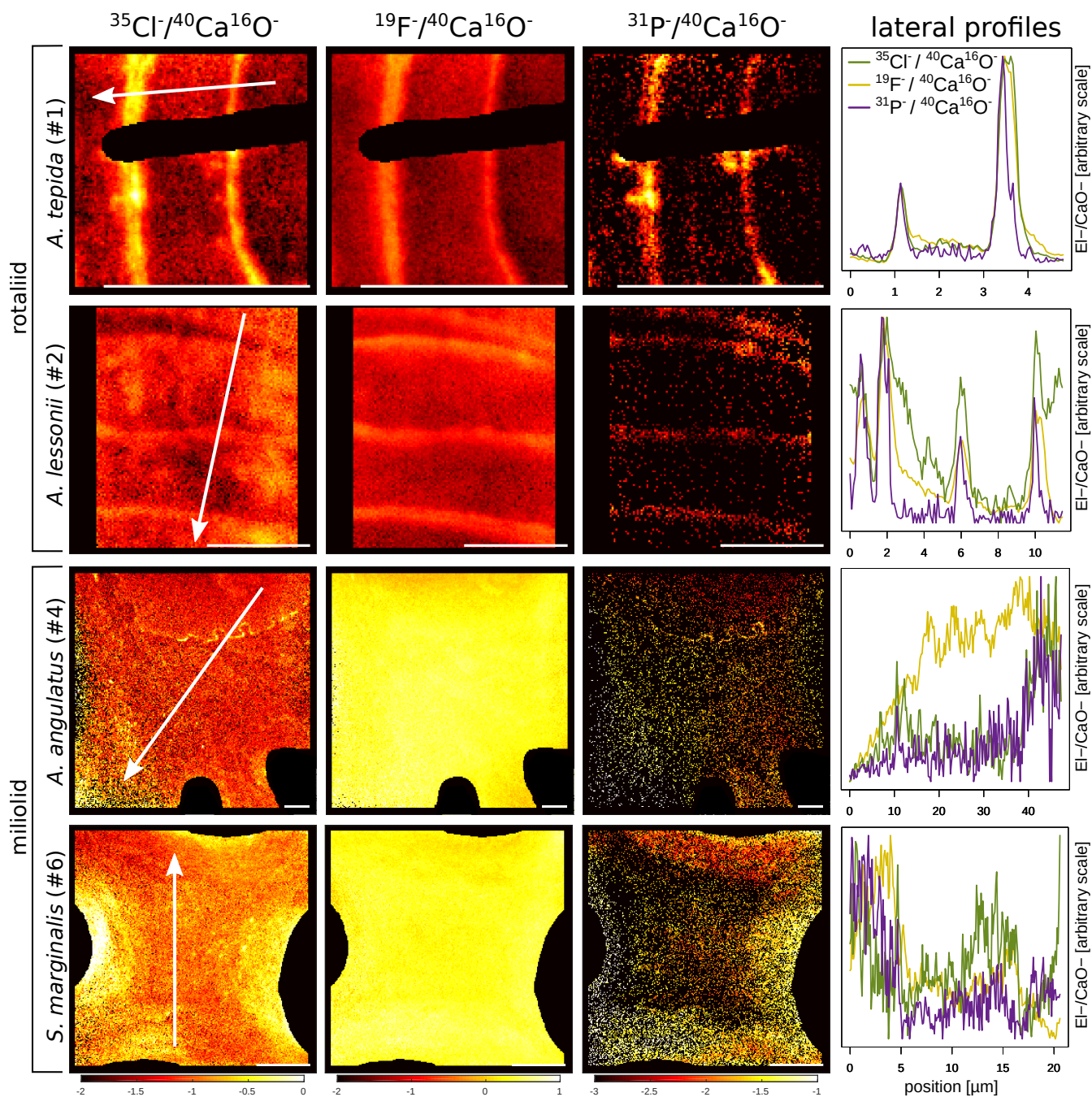
<sup>1</sup> R = rotaliid, M = miliolid.

<sup>†</sup> Selected from the culture experiment of Geerken et al. (2018).

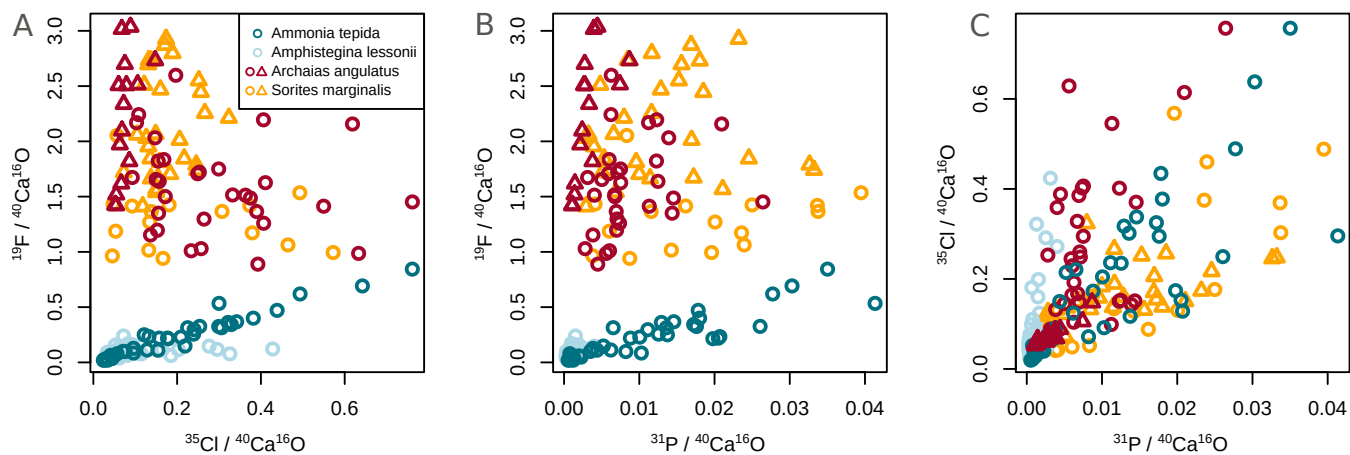
<sup>‡</sup> Selected from the culture experiment of van Dijk et al. (2019).

\* Selected from an unpublished culture experiment (see details in the text).

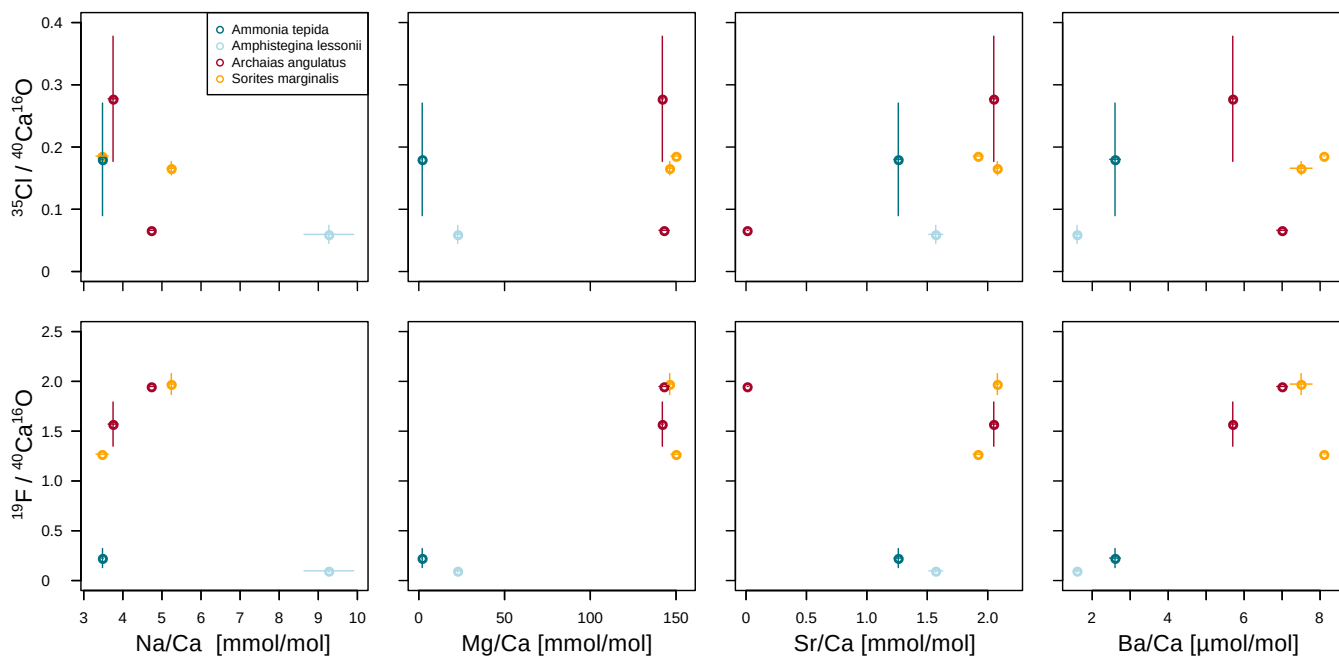




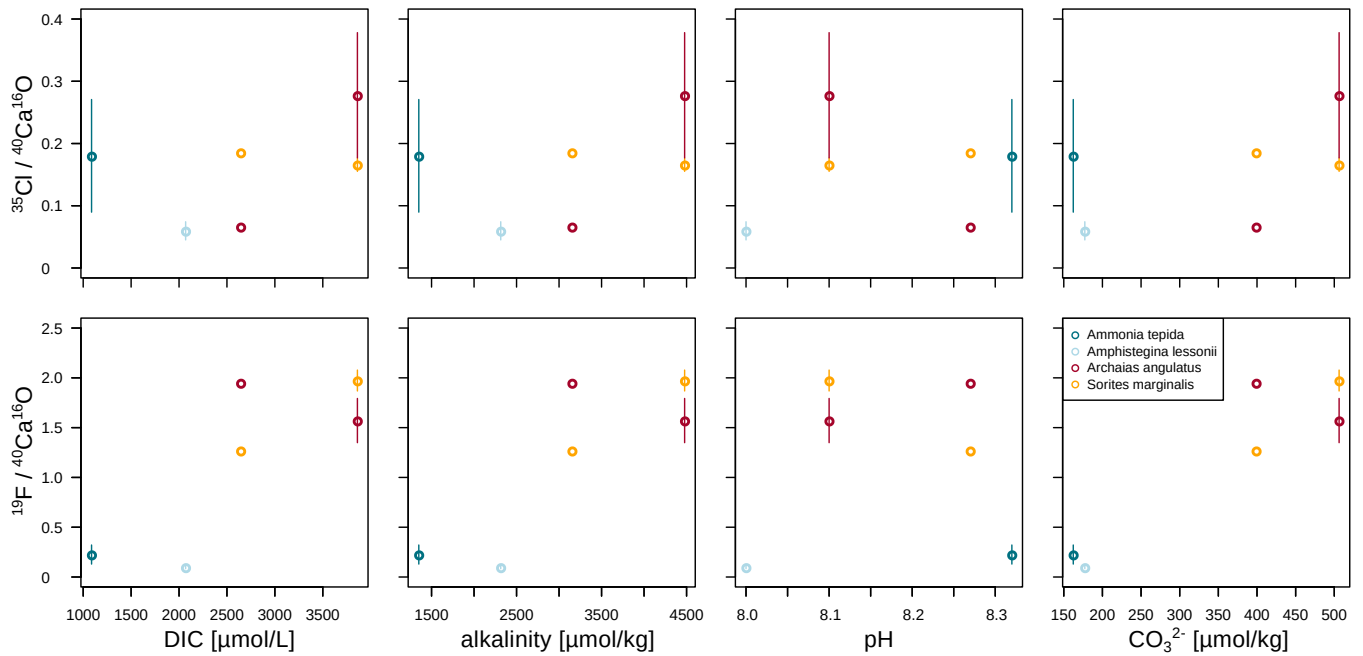
**Figure 1.** Spatial distribution of Cl/Ca, F/Ca, and P/Ca [ion count](#) ratios in the calcite shells of the studied foraminifera species. Shown are representative images as well as lateral profiles along a line going from the inside to the outside of the shell as depicted by an arrow in the images. Note that the displayed ratio images are log-transformed, and that the color-scale for the given ratio is the same for all species. Blacked out areas correspond to resin. The scale bar in each image is 5  $\mu\text{m}$ . [Details on the culturing conditions of these specimens are provided in Table 1.](#) [Locations of the nanoSIMS images within the shells are shown in Figure A1.](#)



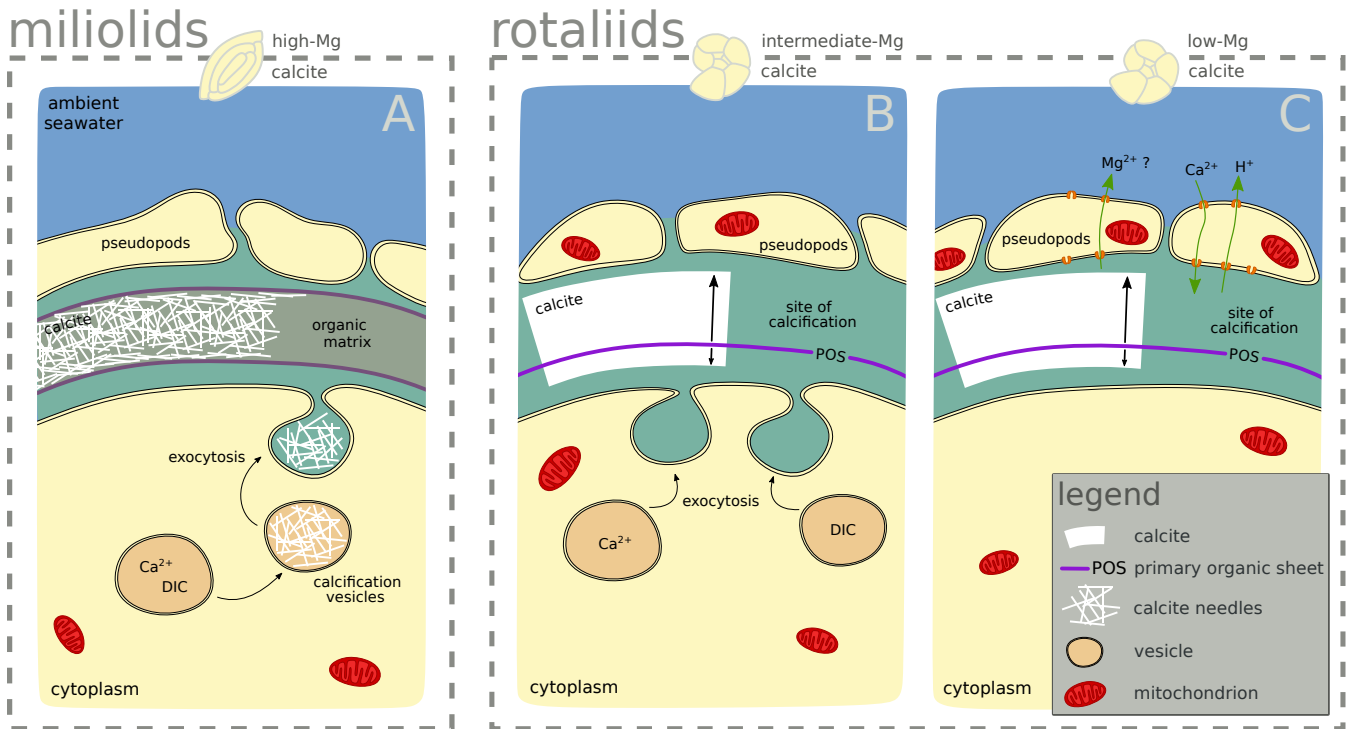
**Figure 2.** Variability of the different  $\text{E}/\text{Ca}$  ratios within shells of the studied foraminifera. Individual data-points represent multiple ROIs drawn on the images as described in Section 2.4. Values are shown as ratios of secondary ion counts measured by nanoSIMS. Symbol shapes with the same colour depict different specimens of the same species.



**Figure 3.** Crossplots of NanoSIMS  $^{35}\text{Cl}/^{40}\text{Ca}^{16}\text{O}$  and  $^{19}\text{F}/^{40}\text{Ca}^{16}\text{O}$  ion count ratios with LA-ICP-MS El/Ca ratios in the same specimen. The error bars depict one standard error of the mean NanoSIMS ion count ratios where more than one image was analysed per specimen, and one standard error of duplicate or triplicate LA-ICP-MS measurements. [Calculation of correlations was not attempted due to the low number of replicate measurements.](#)

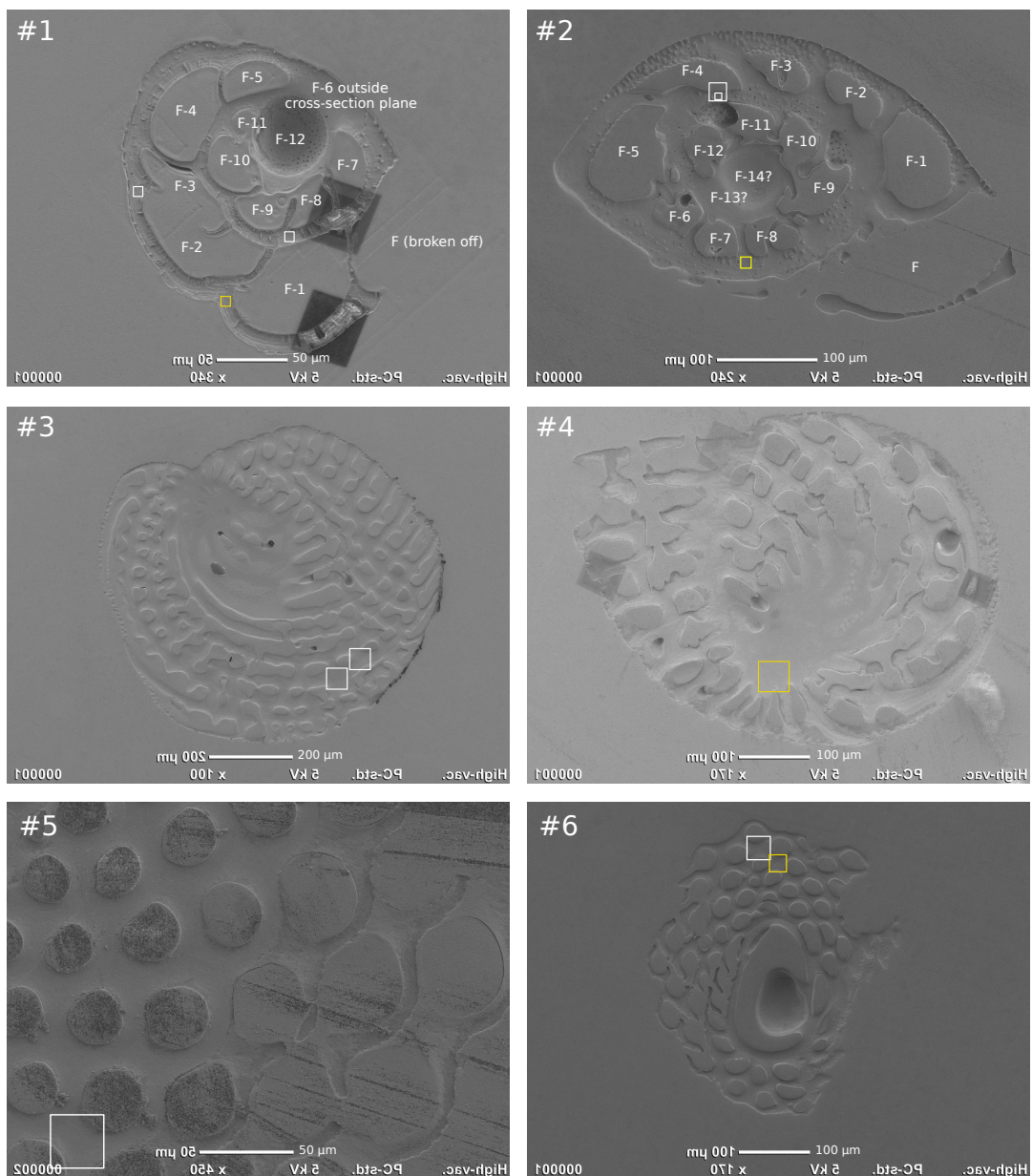


**Figure 4.** Crossplots of NanoSIMS  $^{35}\text{Cl}/^{40}\text{Ca}^{16}\text{O}$  and  $^{19}\text{F}/^{40}\text{Ca}^{16}\text{O}$  ion count ratios with carbonate system parameters of the culture media. The error bars depict one standard error of the mean NanoSIMS ion count ratios where more than one image was analysed per specimen. [Calculation of correlations was not attempted due to the low number of replicate measurements.](#)

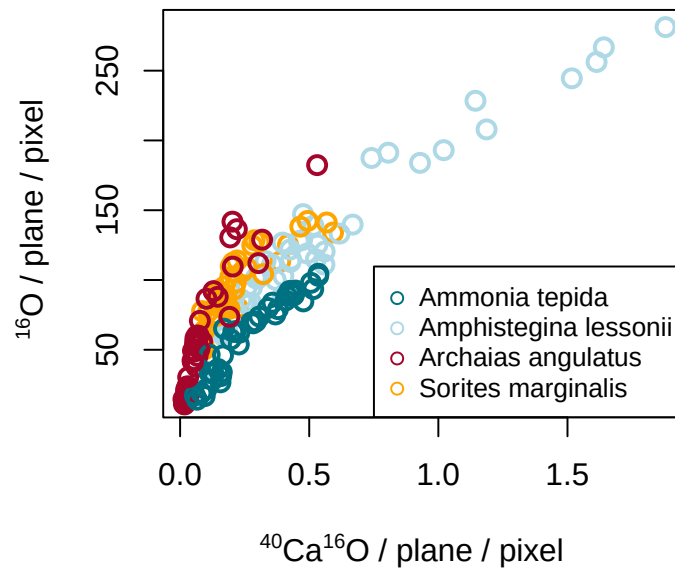


**Figure 5.** Scheme highlighting differences of the calcification mechanisms in miliolid and rotaliid foraminifera. Miliolid foraminifera precipitate calcium crystals in intracellular vesicles prior to arranging them in the shape of the new chamber wall (**AA**; Angell (1980); Hemleben et al. (1986); Debenay et al. (1996, 1998, 2000); Bentov and Erez (2006)). Rotaliid foraminifera precipitate calcite onto organic templates within an extracellular but confined space, and add a new lamella to the entire shell each time they produce a new chamber (**BB**, **CC**; Hemleben et al. (1986); de Nooijer et al. (2014)). In intermediate Mg-calcite producing rotaliid foraminifera (like *A. lessonii*), transport of vesicles to the site of calcification has been observed suggesting controlled biomineralization (at least partly) from internal Ca and carbonate pools (**BB**; de Nooijer et al. (e.g., 2009)). In rotaliid low Mg-calcite producing foraminifera, selective ion transport to the site of calcification via trans-membrane pumping of elements is discussed as the biological control on biomineralization (**CC**; Nehrke et al. (2013); de Nooijer et al. (2014); Toyofuku et al. (2017)).

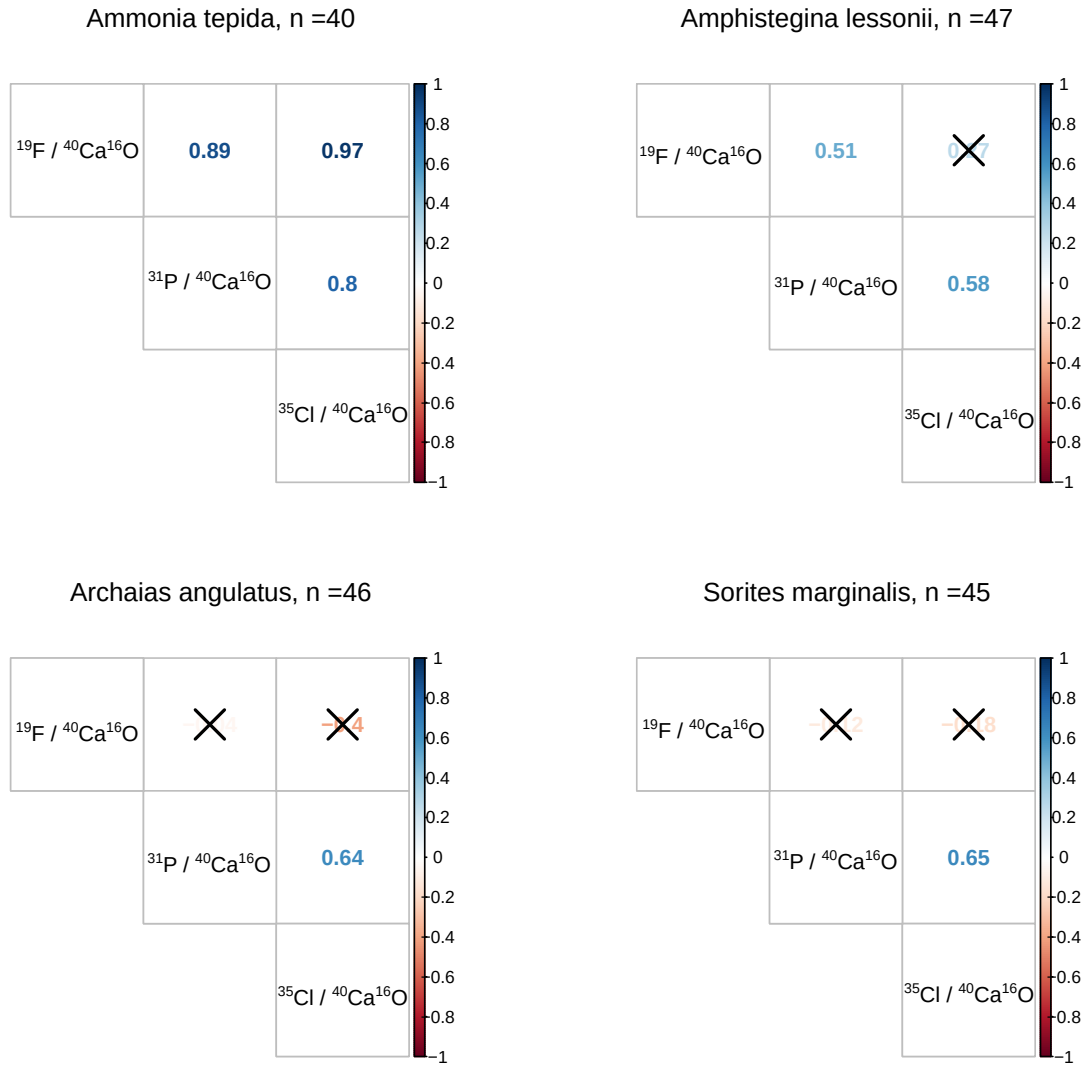
## Appendix A: Supplementary tables and figures



**Figure A1.** SEM images of the analysed specimens with the areas imaged by NanoSIMS indicated by white or yellow squares: *Ammonia tepida* (#1), *Amphistegina lessonii* (#2), *Archaias angulatus* (#3, #4), *Sorites marginalis* (#5, #6). The specimen numbers correspond to those in Table 1 and A1. The yellow squares indicate the locations of the NanoSIMS images shown in Figure 1. SEM images are flipped horizontally to facilitate navigation in the NanoSIMS instrument, where the secondary ion images are horizontally mirrored.

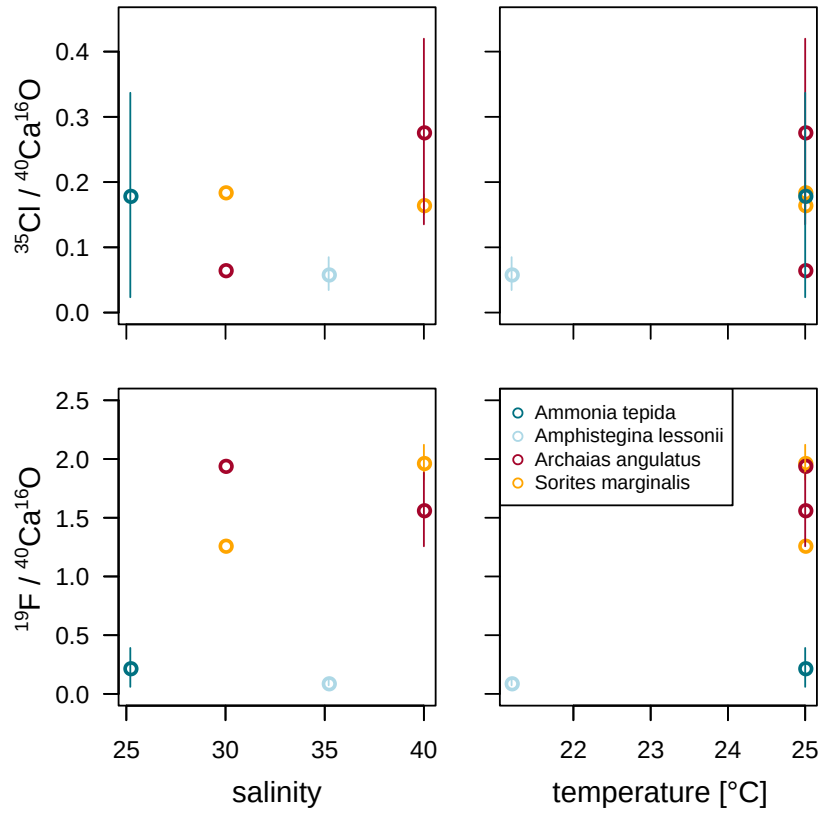


**Figure A2.** NanoSIMS  $^{40}\text{Ca}^{16}\text{O}/\text{plane}/\text{pixel}$  and  $^{16}\text{O}/\text{plane}/\text{pixel}$  in four foraminifera species. The NanoSIMS ion count rates of  $^{40}\text{Ca}^{16}\text{O}$  and  $^{16}\text{O}$  highly correlate in calcite (Figure A2), which is why elemental ratios can be normalized to both for display. Since elemental ratios determined in bulk calcite are normalized to calcium, we present the NanoSIMS data mostly as  $\text{El}/^{40}\text{Ca}^{16}\text{O}$  [ion count](#) ratios.

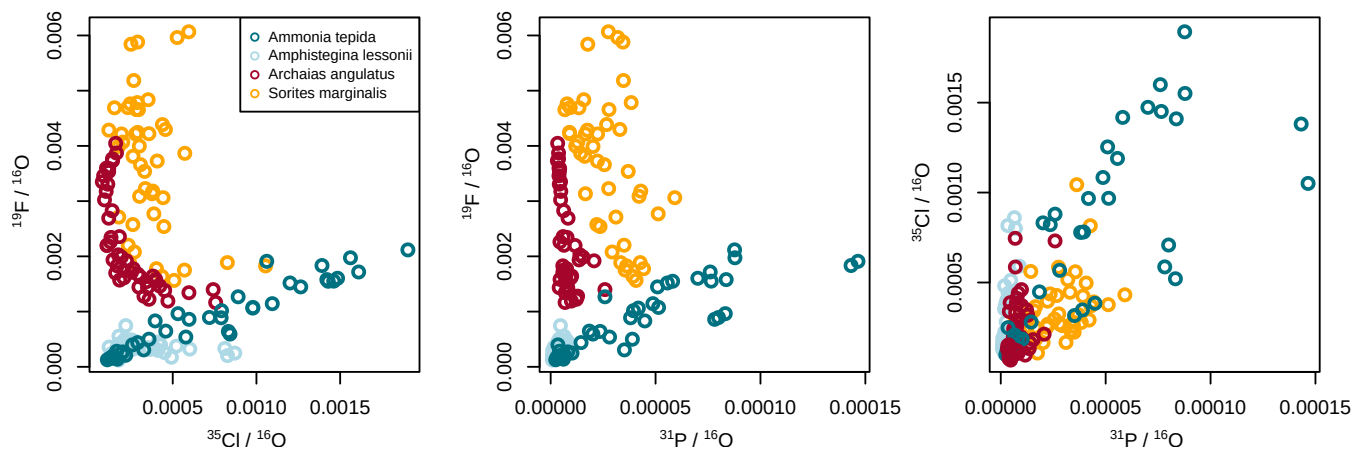


**Figure A3.** Correlation matrices of NanoSIMS  $^{19}\text{F}/^{40}\text{Ca}^{16}\text{O}$ ,  $^{31}\text{P}/^{40}\text{Ca}^{16}\text{O}$ , and  $^{35}\text{Cl}/^{40}\text{Ca}^{16}\text{O}$  ion count ratios per foraminiferal species across n ROIs. Coloured values in the matrix are  $R^2$  and the correlation is significant to a level of  $p < 0.001$  wherever no black cross present. The plot was created using the corplot package in R (Wei and Simko, 2017; R Core Team, 2018).

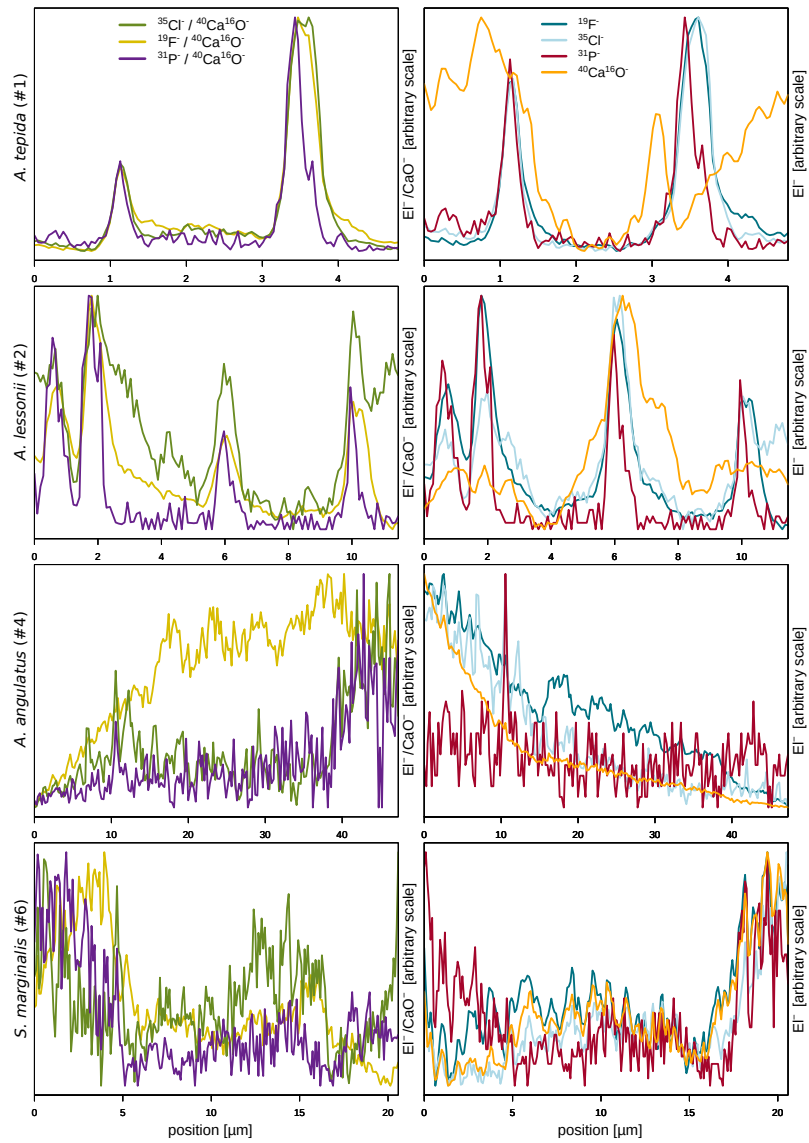




**Figure A4.** NanoSIMS  $^{35}\text{Cl}/^{40}\text{Ca}^{16}\text{O}$  and  $^{19}\text{F}/^{40}\text{Ca}^{16}\text{O}$  ion count ratios in the measured specimen-specimens versus temperature and salinity of the culture media. The error bars depict the standard deviation of the mean NanoSIMS ion count ratios for those specimen-specimens where more than one image was analysed on the shell.



**Figure A5.** Illustration to show that the patterns in figure 2 do not change much when normalizing to  $^{16}\text{O}$  instead of  $^{40}\text{Ca}^{16}\text{O}$ .



**Figure A6.** Illustration to show that the banding pattern seen in the lateral profiles of the rotaliid species in figure 1 is not caused by lower Ca ion counts at the position of organic linings. The left panels show the same lateral profiles of E1/Ca ion count ratios as shown in figure 1, while the right panels show the distribution of the individual elements (shown as ion counts) along the same lateral profile.

**Table A1.** Additional information of specimens. LA-ICP-MS data of average single shell E/Ca ratios were determined on several chambers.

#	specimen ID*	species	symbionts	Na/Ca [mmol/mol]	Mg/Ca [mmol/mol]	Sr/Ca [mmol/mol]	Al/Ca [mmol/mol]	Ba/Ca [mmol/mol]
1	34_61	<i>Ammonia tepida</i>	no	3.48 ± 0.03	2.0 ± 0.1	1.26 ± 0.05	0.009 ± < 0.000	0.0026 ± 0.0002
2	13_110	<i>Amphistegina lessonii</i>	yes	9.27 ± 1.10	22.7 ± 3.8	1.57 ± 0.10	0.008 ± < 0.000	0.0016 ± 0.0001
3	32_89	<i>Archaias angulatus</i>	yes	3.75 ± 0.22	142.0 ± 1.0	2.05 ± 0.06	0.011 ± < 0.000	0.0057 ± 0.0001
4	46_87	<i>Archaias angulatus</i>	yes	4.73 ± 0.22	143.0 ± 4.2	0.01 ± < 0.00	0.008 ± 0.001	0.0070 ± 0.0002
5	01_87	<i>Sorites marginalis</i>	yes	3.47 ± 0.27	150.0 ± 5.2	1.92 ± 0.07	0.153 ± 0.093	0.0081 ± < 0.000
6	30_89	<i>Sorites marginalis</i>	yes	5.24 ± 0.19	146.3 ± 2.5	2.08 ± 0.02	0.044 ± 0.013	0.0075 ± 0.0005

\* Lab internal specimen ID based on stub#\_number. For *A. tepida* as in Geerken et al. (2018).

**Table A2.** NanoSIMS 50L acquisition settings.

NanoSIMS settings*	<i>Ammonia tepida</i>	<i>Amphistegina lessonii</i>	<i>Archaia angulatus</i>	<i>Sorites marginalis</i>
<b>Pre-sputtering</b>				
beam current (in FC <sub>o</sub> ) [pA]	~ 140 (preset 10 in D1–3)	~ 280 (preset 20 in D1–3)	~ 280 (preset 20 in D1–3)	~ 140–280 (preset 10–20 in D1–3)
diaphragm and slits	D1–1	D1–1	D1–1	D1–1
FOV [ $\mu\text{m}^2$ ]	8 × 8	12 × 12 to 23 × 23	55 × 55	25 × 25 to 40 × 40
time [min]	10	10	15	10–15
eGun	on	on	on	on
<b>Image Acquisition</b>				
beam current (in FC <sub>o</sub> ) [pA]	0.5	1	2	1
diaphragm and slits	D1–3, ES–3, AS–2, EnS–1	D1–3, ES–3, AS–2, EnS–1	D1–3, ES–3, AS–2, EnS–1	D1–3, ES–3, AS–2, EnS–1
Detected masses	<sup>12</sup> C, <sup>16</sup> O, <sup>19</sup> F, <sup>31</sup> P, <sup>35</sup> Cl, <sup>37</sup> Cl, <sup>40</sup> Ca <sup>16</sup> O	<sup>12</sup> C, <sup>16</sup> O, <sup>19</sup> F, <sup>31</sup> P, <sup>35</sup> Cl, <sup>37</sup> Cl, <sup>40</sup> Ca <sup>16</sup> O	<sup>12</sup> C, <sup>16</sup> O, <sup>19</sup> F, <sup>31</sup> P, <sup>35</sup> Cl, <sup>37</sup> Cl, <sup>40</sup> Ca <sup>16</sup> O	<sup>12</sup> C, <sup>16</sup> O, <sup>19</sup> F, <sup>31</sup> P, <sup>35</sup> Cl, <sup>37</sup> Cl, <sup>40</sup> Ca <sup>16</sup> O
dwel time [ $\mu\text{s pixel}^{-1}$ ]	1000	1000	1000	1000
image FOV [ $\mu\text{m}^2$ ]	6 × 6	8 × 8 to 20 × 20	50 × 50	23 × 23 to 30 × 30
image resolution [px × px]	128 × 128	128 × 128, once 256 × 256	256 × 256	256 × 256
number of planes	1000	1000, once 250	250	250
eGun	on	on	on	on

\* Abbreviations: objective current (FC<sub>o</sub>); field of view (FOV); electron flood gun (eGun).

## Appendix B: Culture conditions of the foraminiferal species

445 The *A. angulatus* and *S. marginalis* specimens were collected in Sint Eustatius (Oranjestad Bay, 17.479751°N—62.987273°W). The culture experiments with *A. angulatus* and *S. marginalis* were conducted in the same manner as described in van Dijk et al. (2017), with the exception of media preparation. Culture media of different salinities were prepared by mixing natural 0.2  $\mu\text{m}$  filtered seawater with deionized water and 'instant ocean' salt, to obtain a range in salinities between 25–45. The *A. lessonii* specimens are from Burger's Zoo (van Dijk et al., 2019); the culture conditions are reported in van Dijk et al. (2019). The specimens of *A. tepida* were collected on a tidal flat near Den Oever, the Wadden Sea, NL (Hayward et al. 2004); the culture conditions are reported in Geerken et al. (2018).