

Reply to Reviewer 1 (Lennart de Nooijer)

We want to thank Lennart de Nooijer for his comments on our manuscript. His comments and suggestions helped us to improve our manuscript and eradicate mistakes that we overlooked.

Major comments:

Reviewer 1: The authors use their mixing model to explain the difference in Sr/Ca among the foraminifera, but I think the assumed values for partition coefficients may have to be adjusted. Below, I will outline why this is important.

On top of that I think the partition coefficient for Mn is not correct. The D_{Mn} for foraminifera (not from inorganic precipitation experiments; Mucci, 1988) is in the order of 0.5-1; see Van Dijk et al., 2020. *Frontiers in Marine Science* 8: 567701). This D_{Mn} is the one for an intermediate-Mg species (*Amphistegina*), which may well be not unlike that of *H. sarcophaga*. Using this D greatly reduces the difference in Mn/Ca of the foraminifers when assuming that they derive part of their calcifying fluid directly from the host (see figure below). The discussion should consider this smaller difference in Mn/Ca.

+

Similarly, the difference in Sr/Ca becomes (much) smaller if the D_{Sr} is chosen differently. The listed D_{Sr} from Raitzsch et al. (2010) is for *H. depressa*, which is a high-Mg calcite precipitating species. The same paper notes a D_{Sr} of ~0.16 for another species, which would reduce the difference in Sr/Ca by approximately 50%. For *Amphistegina* (see above and e.g. Van Dijk et al., 2017, *Biogeosciences* 14: 497), the D_{Sr} is somewhere inbetween.

Answer: We agree with the reviewer about the problematic distribution coefficient for Mn. We have initially chosen a distribution coefficient from inorganic calcite due to the high range of distribution coefficients reported for benthic foraminifera (Barras et al., 2018; Groeneveld and Filipsson, 2013 and references therein). We have modified the figure to present the results using the distribution coefficients suggested by the reviewer as well as inorganic distribution coefficients (L 411). However, generally speaking the chosen distribution coefficient is not important for the point we are trying to make with this model. What we are trying to show with this model is why we see differences in the foraminifera grown on different hosts regarding Mn/Ca and Sr/Ca but not in the Mg/Ca and Na/Ca ratios, even though the hosts display different compositions.

Reviewer 1: Mechanistically, the model is problematic since there is evidence for selective ion transport over membranes during calcification, so that the contribution of (unmodified) seawater as one endmember (at least for rotallid foraminifera) is unlikely true. I am not against involvement of

seawater *per se*, but for many El/Ca, their ratios in seawater unlikely match those in the calcifying fluid. Secondly, I don't think the El/Ca of the hosts' shells'/ skeletons is directly entering the calcifying fluid of the foraminifer. Rather, the dissolution of the CaCO₃ will alter the [Ca²⁺], [Mg²⁺], etc. in the foraminiferal environment. Especially if the authors see seawater uptake as the main source of ions for calcification, the ions from the dissolution should modify the composition of the seawater surrounding the foraminifera before it affects the foraminiferal site of calcification. Such a less direct connection between the host-derived ions and the composition of the foraminifer's site of calcification will reduce the difference between the two end-members of the model and reduce the difference between the two groups of foraminifera.

Answer: Even if the foraminifera modified the seawater for calcification, which would probably mainly affect the magnesium concentration, this does not change the general outcome of the model and the point we are trying to make with it (See previous comment). Equally there is also evidence for seawater vacuolization without the need for a significant modification of the calcifying fluid (Evans et al., 2018; Bentov et al., 2009). The cited studies were conducted on *Amphistegina lessonii* and *Amphistegina lobifera*, two species that display a chemical composition that is similar to *H. sarcophaga*, at least with regards to Mg and Na (van Dijk et al., 2019; Geerken et al., 2018).

It is impossible with our results to judge if the mixture between host derived material and seawater happen inside or outside of the foraminifera. We think that the transport of dissolved material can happen similar to the transport of organic material to the calcification site, through vesicles and cytoplasm (Spero, 1988). We have added sections, about potential alterations of the vacuolized seawater and potential mixing outside of the foraminifera (L452-4368).

Reviewer 1: Also: unless I am mistaken, for the model outcome to be used as an explanation for the observed Sr/Ca, the ions for calcification have to be largely (>99%) derived from dissolution of the host, which is something the authors should stress and argue that this is likely. I find it unlikely that the foraminifera would need the Ca ions from dissolution of their hosts in order to calcify, given the relatively high seawater Ca-concentrations (at least high compared to the [CO₃₂₋] or the [DIC]).

Answer: We are very sorry, but the statement that ions have to largely derived from dissolution is based on a mistake in our manuscript. Based on the results we expect the foraminifera to gather between 100-1000 mg /l of dissolved carbonate from the host.

Reviewer 1: The authors should be clear about the *reasons* for boring into the bivalve shell/ coral skeleton. I think there are two options, which the authors seem to mingle in their discussion (lines ~230-235). Either dissolving the CaCO₃ is somehow or profitable, or the resulting access to the hosts' insides is profitable. It could also be both, but that cannot be the case for the same reason. This

means that they do not likely extract pre-concentrated fluid for their Ca-need and at the same time dissolve the CaCO_3 for their Ca-need. Given the specific tunnel made in the *Acesta*, it does not look like that dissolving the shell in itself is the main business of the foraminifera. Rather, the tunnel seems to be made to provide access to something that the foraminifer is after. This may suggest that the dissolved Ca, DIC and other ions may at best have an 'unintentional' effect on the foraminiferal shell chemistry. Despite the speculative nature of this discussion, I hope that the authors can spell out more clearly what may be and what may not be happening when dissolving and calcifying.

Answer: In *A. excavata*, tissue damage is reported in the mantle tissue close to the boring (Cedhagen, 1994). Therefore, we can be rather sure that the foraminifera feeds on the bivalve's soft tissue. However, we don't see why the foraminifera should not benefit from the extrapallial-calcifying fluid (ECF), which is also very nutritious (Yin et al., 2005) and additionally can provide Ca and CO_2 to aid calcification (Crenshaw, 1972). Feeding on extra pallial fluid of bivalves was also shown in *C. refulgens* with labeled tracer experiments (Alexander and Delaca, 1987). Amino acids in the mantle fluid are highly concentrated (2.5 mmol) and suffice to satisfy the foraminifera's max influx rate (Alexander and Delaca, 1987) The attachment etchings are considered to help the foraminifera to anchor on their host, but the host have no effective way of removing the parasites. *H. sarcophaga* is also reported to infest Leptochitonidae, where the etching activity is so intense that the foraminifera sometimes break the hosts shell and fall of as a consequence (Sigwart, 2009). This leads us to assume that there must be other reasons for the large etchings other than attachment. Experiments with labeled Ca would be great to show, what ends up in the foraminifera. We added a section that summarizes what is known about the boring and etching process including the processes used for etching, according to the reviewer's recommendation (L.351-373).

Reviewer 1: Related to this, I think the discussion would benefit from outlining the possible mechanism of dissolution by these foraminifera. Normally, I try not refrain from promoting my own work when reviewing, but in this case, the reduction of environmental pH by the foraminifer (Toyofuku et al., 2017) may provide the mechanism that foraminifera employ when dissolving calcium carbonate. There is similar work on the mechanism of bio-eroding sponges showing that pH regulation is the basis of bio-erosion by sponges (e.g. Webb et al., 2019; Scientific Reports 9: 758). Interestingly, excavating sponges also can trigger calcification in their hosts: this similarity is maybe something to mention in the discussion as well.

Answer: We want to thank the reviewer for this suggestion and suggested papers. We added a section about possible mechanisms utilized for boring by the foraminifera (L.367-373)

Reviewer 1: Finally, what I miss in the manuscript is a discussion on the elemental composition of these species as such. Compared to other species, the Mg/Ca and Na/Ca are relatively high. If I am

correct, all intermediate- or high-Mg/Ca Rotallid species are larger tropical foraminifera. That this is a nonphotosynthetic symbiont-bearing species, from another family than Sorites, Amphistegina, Heterostegina, Marginopra, etc. is interesting in itself.

Answer: We added a short chapter, discussing the high Mg/Ca ratios we measured in *H. sarcophaga* in comparison to other foraminifera species(L.583-607)

Minor comments:

Reviewer 1: Line 39/40: I am not sure parasitism as such is a 'feeding mechanism'. Without going into abstract discussions, parasitism is more a (symbiotic) relationship between organisms. Parasitism is not of the same 'level' as e.g. grazing. More accurate would be something like '...or their feeding mechanism is related to a parasitic lifestyle.'

Answer: The reviewer is completely right that parasitism is not a proper word to describe a feeding mechanism. We changed the wording to "parasitic feeding" (L.39)

Reviewer 1: Line 55: what does SRZ mean?

Answer: SRZ means shell repair zone (L.210), meaning the area where the bivalve repaired the foraminifera's boring by forming a calcified callus sealing. We added an explanation to L56.

Reviewer 1: Line 64: given the balance between Ca and DIC in seawater, it may be more likely that dissolution of the *Lophelia* serves the need for DIC rather than Ca to calcify by the foraminifer.

Answer: That is also possible. We added this information (L.65)

Reviewer 1: Line 221: 'specimens

Answer: Corrected

Reviewer 1: Figure 2: could you swap the results from the aragonite and the calcite of the *A. excavata* in all panels? Then the order (calcite/ aragonite/ SRZ) matches the layers as they are deposited

Answer: Good suggestion. We changed the figure according to the reviewer's suggestion (L.220).

Reviewer 1: Line 234: add ')

Answer: Corrected

Reviewer 1: Line 256: please italicize '*L. pertusa*', 'lower' should be 'more depleted'

Answer: Corrected

Reviewer 1 : Figure 4: it is confusing that two samples have a similar color (blue), while they are unrelated. I can imagine that the samples that have something in common (e.g. the HL and the *Lophelia*) have a similar shade.

Answer: We changed the colors of the figure but we refrained from using similar colors for related samples because we think it makes the distinction between samples harder (L.274).

Reviewer 1: Line 337: I think the paper of Schleinkofer et al. (2021) does list the Sr/Ca of *Lophelia*, but not that of *Acesta*

Answer: This citation was a placeholder for an accepted manuscript that awaited data upload to Pangaea. It is fully available now and properly cited in the reference list.

Reviewer 1: Line 345: the mixing model is not really a further investigation into the mechanisms, but rather an exploration of the consequences of the assumptions.

Answer: We changed the wording to read: “In order to further investigate the observed results,.....” (L.393)

References:

Alexander, S. P. and Delaca, T. E.: Feeding adaptations of the foraminiferan *Cibicides refulgens* living epizoically and parasitically on the Antarctic scallop *Adamussium colbecki*, 173, 136–159, <https://doi.org/10.2307/1541868>, 1987.

Barras, C., Mouret, A., Nardelli, M. P., Metzger, E., Petersen, J., La, C., Filipsson, H. L., and Jorissen, F.: Experimental calibration of manganese incorporation in foraminiferal calcite, 237, 49–64, <https://doi.org/10.1016/j.gca.2018.06.009>, 2018.

Bentov, S., Brownlee, C., and Erez, J.: The role of seawater endocytosis in the biomineralization process in calcareous foraminifera, 106, 21500–21504, <https://doi.org/10.1073/pnas.0906636106>, 2009.

Cedhagen, T.: Taxonomy and biology of hyrrokkin sarcophaga gen. Et Sp. N., a parasitic foraminiferan (rosalinidae), 79, 65–82, <https://doi.org/10.1080/00364827.1994.10413549>, 1994.

Crenshaw, M. A.: The Inorganic Composition Of Molluscan Extrapallial Fluid, 143, 506–512, <https://doi.org/10.2307/1540180>, 1972.

van Dijk, I., Mouret, A., Cotte, M., le Houedec, S., Oron, S., Reichart, G. J., Reyes-Herrera, J., Filipsson, H. L., and Barras, C.: Chemical Heterogeneity of Mg, Mn, Na, S, and Sr in Benthic Foraminiferal Calcite, 7, 281, <https://doi.org/10.3389/feart.2019.00281>, 2019.

Evans, D., Müller, W., and Erez, J.: Assessing foraminifera biomineralisation models through trace element data of cultures under variable seawater chemistry, 236, 198–217, <https://doi.org/10.1016/j.gca.2018.02.048>, 2018.

Geerken, E., Jan De Nooijer, L., van Dijk, I., and Reichart, G. J.: Impact of salinity on element incorporation in two benthic foraminiferal species with contrasting magnesium contents, 15, 2205–2218, <https://doi.org/10.5194/bg-15-2205-2018>, 2018.

Groeneveld, J. and Filipsson, H. L.: Mg/Ca and Mn/Ca ratios in benthic foraminifera: the potential to reconstruct past variations in temperature and hypoxia in shelf regions, 10, 5125–5138, <https://doi.org/10.5194/bg-10-5125-2013>, 2013.

Sigwart, J. D.: Parasitic foraminifers on a deep-sea chiton (Mollusca, Polyplacophora, Leptochitonidae) from Iceland, 5, 193–199, <https://doi.org/10.1080/17451000802266641>, 2009.

Spero, H. J.: Ultrastructural examination of chamber morphogenesis and biomineralization in the planktonic foraminifer *Orbulina universa*, 99, 9–20, <https://doi.org/10.1007/BF00644972>, 1988.

Yin, Y., Huang, J., Paine, M. L., Reinhold, V. N., and Chasteen, N. D.: Structural Characterization of the Major Extrapallial Fluid Protein of the Mollusc *Mytilus edulis* : Implications for Function †, 44, 10720–10731, <https://doi.org/10.1021/bi0505565>, 2005.