

Interactive
Comment

Interactive comment on “Physiological controls on seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*” by L. J. de Nooijer et al.

Anonymous Referee #1

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This is an interesting paper trying to shed more light on the process of biomineralization in foraminifera using fluorescent laser confocal microscopy. The authors use the species *Ammonia tepida* (a shallow water coastal species) that may serve as a good model for the perforate foraminifera. They have used 2 fluorescent dyes: FM1-43 that adheres to membranes and Fluo3-AM that indicates the concentration of free Ca²⁺ in the cytosol. In addition they used fluorescent latex-beads (0.5 μm diameter) that probably indicated the process of phagocytosis.

They observed very low cytosolic Ca²⁺ concentrations (as expected for all cells) and also some intracellular vesicles with higher Ca²⁺ concentrations. The beads were in-

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deed ingested and the membrane dye showed interesting thread like structures that in one case looked like a complete circle. They also observed occasional small vesicles ($3 \mu\text{m}$). While these observations are interesting and worth while reporting, their interpretation is not clear. There is no straightforward connection between these observations and the process of chamber formation and/or calcification. The observational part of the manuscript is very modest (as can be seen from the short results section), whereas the discussion is overstretched to almost a review of calcification in foraminifera, portraying little connection to the observations in some parts and lacking proper credit to related work.

Our main criticism is therefore on the discussion. They talk about seawater vacuolization as a source for ions for calcification and claim to be the first ones to demonstrate it. This is obviously not justified since Erez (2003) proposed it based on observations using different membrane impermeable dyes. It would have been nice if they could verify Erez's model for another species, which is symbiont barren. But their claim for direct evidence of vacuolization of seawater is unjustified in view of their inconclusive data. Labeled membranes do not necessarily show vacuoles but rather pseudopodial networks. In any case these networks are not showing any direct connection to calcification. The occasional vesicles are also not a direct proof. Such small vesicles in the calcification zone are reported by almost every TEM study of foraminiferal calcification, but their function is unclear. Using the fluorescent beads they were able to show normal phagocytosis which is the process by which foraminifera feed on particles (algae). So this again is not directly connected to calcification. With respect to Ca^{2+} , they imaged its cytosolic concentration to be very low as expected. They also observe free Ca bound by Fluo-3 within vesicles, but state that $>90\%$ of the Ca-containing vesicles are not transported towards the site of calcification. All their discussion concerning Ca budget is basically similar to calculations and assumptions that were given in the review of Erez (2003) based on experiments with ^{45}Ca . The authors conclude that Ca transported by vacuoles (or vesicles) does not suffice for new chamber formation (even if 100% of the foraminifera's inner volume was assumed to store Ca-containing

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vesicles, which is apparently not the case). The following discussion on ACC as a means of internal Ca storage would benefit from Bentov and Erez (2005, *Geology*, 23(11): 841-844), who observed cytoplasmic Ca containing granules confined in vesicles in live specimens of *A. lobifera* as well as other benthic and planktonic species. The Mg/Ca discussion is basically a short repeat of what was reviewed by Bentov and Erez (2006), but they do not cite this paper in this section. The entire discussion on "Inorganic carbon utilization" is a repeat of the comprehensive discussion in Erez (2003) and the references cited in that paper. The data shown in the present manuscript has no relevance to this issue and hence it should be taken out.

In summary there is a major difference between the elaborate discussion (which is logical for most of what they say) and the poor data set. Almost nothing in their data supports their discussion. Hence our recommendation is not to publish this paper in its present form. The authors should correct their manuscript according to the above comments. The discussion should be shortened to about 30% of its size (they can include the Ca²⁺ budget) but all the rest is a literature review that is not relevant.

More technical comments:

Introduction

1.Pg 7085 Line 26 "calciumcarbonate" separate to 2 words also throughout the manuscript

2.Pg 7086 Line 1: The range in MgCO₃ in foraminifera is more like 0.2-20 mol%.

3.Pg 7086 Line 5: Specify that the pH increase is "internal" and give reference. Methods and Results

4.Did you run control with 5% DMSO? If not, perhaps the whole behavior of the organisms was not normal. This needs to be thoroughly checked.

5.Explain what the fluorescent probes are for.

6. What is the initial diameter of the individuals chosen for the experiments? From the model, we assume it is $\sim 100 \mu\text{m}$.

7. The authors state that 90% of the free Ca bound by Fluo-3 within vesicles do not participate in calcification. Was this value calculated quantitatively? If so, how?

8. They observe that at most 20 Ca-containing vesicles are being transported to the site of calcification during the whole chamber building process. The authors should assure the reader that imaging resolution was not an issue by comparing the frequency of their imaging (every minute) with the duration of the calcification process (100-200 minutes?).

Figures

Figure 1:

9. The authors should show the transparent channel images to demonstrate exactly the CaCO_3 relative to the fluorescent dye distribution. They have done it with a line drawing but it needs a real image

10. It is hard to visualize the space that the cytoplasm takes up as “thin films” in the last chamber (Re: page 7088 lines 23-24). A picture would help to illustrate this point. Otherwise, the description should be made more precise.

11. Please mark the direction of the aperture as well as the radial structures (Re: page 7089 line 1) in Fig. 1g.

Figure 2:

12. Can the authors mark the protective cyst more clearly in Fig 2? What are the 3 fluorescent spheres with much bigger diameter than the beads in Fig 2?

13. Please correct the reference as Gebauer et al., 2008.

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