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Biogeosciences Discussions

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

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Dear Dr. Toporski,

We were pleased to receive two constructive reviews regarding our manuscript entitled "Physiological controls on seawater uptake and calcification in the benthic foraminifer *Ammonia tepida*". We have incorporated their comments to improve our manuscript and would like you to consider this adjusted manuscript for publication in *Biogeosciences.* Below, we discuss all comments and concerns in detail.

Regards,

Lennart de Nooijer

Reviewer #1

Major comments

We do not agree with this reviewer that our discussion is not supported by the results that we present. The calcification pathway of *Ammonia tepida* is hardly investigated. The recent progress in culturing this species (Dissard et al., 2009a; 2009b, 2009c; Raitzsch et al., submitted; Duenas-Bohorquez et al., in prep.; De Nooijer et al., 2007; De Nooijer et al., in prep.) allows for the very important verification of the so-called 'general calcification pathway' for perforate foraminifera (Erez, 2003; Bentov and Erez, 2006). The results that these models are based on, are derived from several species and for some of these claims, reults are inaccessible since they are only reported as conference abstracts (e.g. claims on the existence of ATP-driven Ca-pumps). For these two reasons, we think that both the results and discussions are novel and important.

However, we do agree with some of the suggestions provided by reviewer #1 and have added a dataset to our manuscript on fluid-phase endocytosis in *A. tepida*. Following the report on the uptake of FITC-dextran in Erez (2003), we incubated individuals of *A. tepida* with this fluorescent dye to track possible uptake of seawater, rather than phagocytosis of the fluorescent beads. Surprisingly, FITC-uptake did not follow the pattern as reported in the abstract of Bentov and Erez (2001), summarized in Erez (2003). The FITC-dextran complex was taken up into the outer chambers of the individual (indeed proving fluid-phase endocytosis), but did not lead to the formation of fluorescent vesicles within the cell. Rather, the FITC-dextran taken up was egested rather quickly, suggesting a slightly different calcification mechanism for *A. tepida* than for that reported by Erez (2003). This lead to the addition of one figure and the description on FITC-uptake. Furthermore, section 4.1 of the Discussion was adjusted to accomodate these observations and its relation to previous reports.

We also agree with this reviewer that the discussion on inorganic carbon utilization is

not well supported by our data and therefore removed this from the discussion (formerly section 4.4). We also shortened the rest of the Discussion.

Minor comments:

1. "calciumcarbonate" is separated into two words throughout the paper.
2. Foraminiferal Mg content is now stated to lie between 0.2 and 20 mmol/mol.
3. Altered pH is internal (De Nooijer et al., 2009).
4. As we showed in (Toyofuku et al., 2008), small concentrations of DMSO do not visually affect pseudopodial movements/ chamber formation in *Ammonia spp*. With more of the Fluo3-AM experiments done, specimens are regularly observed to reproduce in the presence of DMSO (used to dissolve the Fluo3-AM). Therefore, we do not consider DMSO to affect foraminiferal physiology considerably. We have restated this in the revised manuscript (first paragraph of 2.2).
5. We already stated that the fluorescent probes target cell membranes (FM1-43) and free Ca^{2+} (Fluo3-AM). Latex beads do not target anything specific, but cannot cross cell membranes and their uptake is therefore indicative of endocytosis. The latter information is added to the manuscript.
6. The individuals we used to incubate consisted of 3-5 chambers, corresponding roughly to 100 micrometer in diameter (see figures 2 and 4). This was added to the end of section 2.1.
7. In figure 3, many Ca^{2+}-containing vesicles are still present in the cell after chamber formation is complete (see also Toyofuku, 2008). The 90% is a rough estimate, and only used to put the observations in perspective. Since the exact amount is very difficult to determine, we kept this value and preceded it with a

word of caution ('Although difficult to determine precisely, approximately ...'; section 3.3).

8. Since most of the vesicles that are moving through the cell are recognizable over multiple scans made 1 minute after one another, individual vesicles can often be tracked from picture to picture. In that way, we found that the transport to the site of calcification was often estimated to be several minutes (e.g. from the F-3 to the F cahmber). Coresponding to the app time of 1 minute that it takes for a high-pH vesicle to be transported from F-1 to the final chamber (De Nooijer et al., 2009). This was briefly added to the adopted version (section 3.3).
9. We decided to keep the drawing in figure 1 because overlying the fluorescent image will severely deter the quality of the image. The alternative is to show the transparent channel in addition to the fluoerscent one, but this does not allow a direct comparison of the fluorescent signal and the position of the chamber wall(s) and therefore adding the transparent channel will not add information.
10. The descriptions on the thin films (Results, section 3.1) was extended somewhat to clarifyt the observations.
11. Subfigure 1G was adjusted and now includes an arrow to indicate the position of the aperture (outside the scanned area). Caption was adjusted accordingly.
12. The protective cyst, containin the fluorescnt clusters was marked more clearly (Figure 2) and the caption was changed to underscore that the clusters are part of the cyst.
13. This reference was corrected to Gebauer et al., 2008.

Reviewer #2

All comments made by this reviewer were incorporated into the revised manuscript. Most of them resulted in alterations that we do not spell out here since they either literally follow the suggestion or were covered in our reply to the first reviewer. The more substantial changes include the following:

1. The introduction was altered so that the first three sentences were removed and one was added to state that the production of a new chamber may well involve an amorphous precursor.
2. We made sure to adopt American English throughout the manuscript.
3. The introduction was shortened by combining the first two paragraphs and deleting app. half of the sentences.
4. We did not include precise observations concerning reproduction rates and such: will be discussed in a following paper since these are currently being established at different conditions.
5. CLSM was briefly explained.
6. The fact that latex beads cannot cross cell membranes and its implication (i.e. uptake being the result of endocytosis) is mentioned in the Methods.
7. We have no direct observations concerning the suposed ACC-cluster and therefore not included any ideas on the size of them.
8. Figure 2 has changed so that the new chamber is indicated. The beads outside the foraminiferal individual were already mentioned but the caption now includes 'clustered' to avoid confusion.
9. Figure 5 (formerly figure 4) has changed somewhat, but we decided not to include organic linings, calcium pumps, etc since their presence and location does not

follow from our experiments and are already depicted in the summarizing picture in Bentov and Erez (2006).

Interactive comment on Biogeosciences Discuss., 6, 7083, 2009.