

Interactive comment on “Technical Note: Towards resolving in situ, centimeter-scale location and timing of biomineralization in calcareous meiobenthos – the Calcein-Osmotic pump method” by J. M. Bernhard et al.

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We thank the anonymous reviewer for their review and perspective.

Regarding the reviewer's “many questions”, we provide the following responses. The actual concentration of calcein loaded in our osmopumps was 100 mg per liter. That concentration can be varied, although considerably higher concentrations have not been tested. The diffusion rate in the environment depends on the temperature, salinity (osmolality), and sediment properties (water content, connectivity, grain size, forma-

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tion resistivity). The rate of diffusion in the environment depends on the temperature, salinity, etc. The horizontal and vertical distance that the calcein solution dispenses depends on the sediment porosity, grain size, sorting, formation resistivity factor, and connectivity as well as current speeds and the benthic community and its bioturbation rate(s). The length of time that calcein is dispensed depends mostly on the temperature and salinity (osmolality), as well as the configuration (model and size) of the osmopump.

We feel that taking the time to do more calcification experiments with different sediments from different settings is beyond the scope of this Technical Note. As the reviewer admits, the radius of dispersion and timing of dispensation will depend on specific physicochemical conditions (i.e., specific sediment grain sizes, rounding, sorting, temperature, porosity, salinity, etc). We feel we have demonstrated that the method works in two settings: sands for clams and muds for benthic foraminifera. If the paper were hypothetical, then we would not have shown any images. We agree that it will be necessary for people who wish to employ this method to “do their homework”, determining the radius of dispersion and the length of dispensation, depending on exactly what their questions are. For some, however, it may be relevant to merely show that some specimens calcified in a particular setting or how fast specimens grew. It may not be necessary for all researchers to require quantification of the entire population.

A recently finished thesis project at Lund University (MSc student Susanne Landgren) confirms the practical use of the osmopumps using foraminiferal-laden fjord sediments (Landgren S., 2015, Dissertations in Geology at Lund University, report no 431, <https://lup.lub.lu.se/student-papers/search/publication/5425178>). Further, we presented initial results at a recent meeting (Landgren S., Filipsson, H.L., Charrieau, L., Bernhard., J.M: In situ biomineralisation of benthic foraminifera during hypoxic and normoxic conditions. The Micropalaeontological Society, Foraminifera and Calcareous Nannofossil Groups, Spring Meeting 2015, June 14- 18, 2015, Plymouth University, Plymouth, UK). A manuscript describing those results is in preparation and will be sub-

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mitted sometime this autumn, but the present Technical Note focuses as an introduction of the method because Bernhard developed the method in her lab. The first true field application was by Filipsson's lab, and thus will be led by her student Landgren. Further, Susanne developed a matlab script to calculate the dispensation time; this will be presented in her publication. Further discussion of diffusion gradients is presented in our response to Reviewer 1, Lennart de Nooijer.

Regarding the title, which begins with the phrase "Towards resolving", it clearly states that this is a method that will help establish location of calcification. We do not claim anywhere that all details are resolved.

Regarding specific comment 1, we feel the Introduction provides context so we do not feel compelled to shorten that aspect of the Introduction. Biomineralization in foraminifera is the focus of considerable research at this time (e.g., de Nooijer et al., 2014 Earth Science Review; Bentov et al. 2009 PNAS; Nehrke et al., 2013 this journal), however, as far as we are aware none has done in situ biomineralization experiments, and this one reason is why this method might prove to be particularly useful to some investigators.

Regarding specific point 3.3 (potential applications), we appreciate the suggestion to include discussion about potential of additional osmopump-dispersed fluorescent or isotopic markers. We prefer to refrain from adding discourse on this topic because we have not tested any additional compounds. We assume that people can develop their own applications, but of course warn that they will have to demonstrate proof of concept. While CellTracker Green could be dispensed from the osmopumps, in most cases, core collection proves adequate for such applications, given the relatively short (6-16 hour) incubations required of that probe. We are not confident that using isotopically labeled nitrate, for example, would work as we do not know if an adequately concentrated solution could be made.

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