

Interactive
Comment

***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.**

Anonymous Referee #2

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General comments A paper by Bernhard and others introduces a new method to study microhabitats and biomineralization in calcareous microorganisms in situ, using a combination of small osmotic pumps and the fluorescent marker calcein. Osmotic pumps are commercial products and calcein has been widely used for biomineralization of calcareous organisms, but a combination of these two is novel and worth reporting. This method would be particularly useful for deep-sea biology.

However, although I understand this paper is a “technical note”, I think supporting data are necessary to assess if this method is really useful or not. I have many questions re-

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mained after reading this paper; How much is the actual concentration of calcein? How fast is the diffusion rate in the environment? How far is calcein solution really reached horizontally and vertically? How long does calcein really disperse? I understand this would be dependent upon substrate types and sediment properties (grain size, porosity, etc.). Authors should present verification tests under several different settings (e.g, mud and fine sand typical for deep sea and shelf setting, respectively). Without such supporting data, this method is so hypothetically based that we cannot assess whether unstained organisms either indicate no calcification during experiments or is due to a low concentration and narrow dispersion range of calcein. Therefore, based on the present version of the paper, I doubt this method is really resolving in situ centimeter-scale location and timing of biomineralization, as shown in the title of this paper. I suggest authors take into consideration these comments for the final version of the manuscript.

Specific comments 1. Introduction: this section mainly describes significance in studying biomineralization and microhabitats of calcareous microorganisms as proxy for paleoceanography. However, most of the contents are not directly related to the method shown in this paper, and should be summarized. I would rather like to know more about previous studies of in situ experiments of biomineralization and microhabitats if any.

3.3. Potential applications: I think the osmotic pumps are useful together with other fluorescent and isotope markers. Authors should consider potential applications of osmotic pumps with other markers.

Technical corrections This paper is well written and structured. I could not find any typing and grammatical errors in the paper.

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