CSI Microscale: Calcification Scene Investigation of calcareous microbenthos -- Method development and test case

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Project summary

Because of their small size and inhabitation of deep-sea sediments, understanding when and where calcareous microbenthos form their skeletons is not easily pinpointed. Thus, we liken this task to the popular CSI television franchise, where Crime Scene Investigators use a barrage of high-tech methods to solve their assigned cases. Wearing research-detective hats, we proposed to unravel this mystery by developing a new method that would unequivocally identify where and when important calcareous microfauna such as benthic foraminifera calcify *in situ*. Chemical signatures in the calcium carbonate shells of benthic foraminifera are arguably the most commonly used tool in paleoceanography; not knowing where and when such microfauna calcify introduces significant uncertainty in paleoceanographic and paleoclimate data.

Specifically, we proposed to develop a method to fluorescently label calcite as it was precipitated in benthic foraminiferal shells while they were living in sediments using osmotic pumps to slowly dispense calcein, a fluorescent compound incorporated into carbonate shells precipitated during such incubation. Osmotic pumps are devices designed to deliver drugs to animals; as originally designed, they are installed in an animal under their skin. We, obviously, use the osmotic pumps in a completely different manner: we fill osmotic pumps with a fluorescent liquid and place them in marine seafloor sediments to allow the osmotic pump contents to disperse into the sediments.

To obtain sediments containing calcareous microfauna (benthic foraminifera) for our experiments, we collected twice. In November 2011 we had a day trip on the WHOI vessel *Tioga*. In May 2013, we had a 3-day cruise on RV *Endeavor*. The *Endeavor* cruise was funded by another (NSF) project, for unrelated purposes. Material for our CSI Microscale investigations was collected from a site south of Martha's Vineyard. This site, the Mud Patch, is on the broad continental shelf (40°30'N, 70°45'W), at a depth of approximately 80 m. The site is well known as being an area where currents collide, so sediments drop out of the water column in this area, causing it to be muddy (thus the name).

Cores were brought back to the Bernhard laboratory's cold room (housed in the Stanley W. Watson building on Quissett Campus). For our first experiment, we placed the osmotic pumps into the cores so that the point source was at a known depth below the sediment water interface (Figure 1). After ~4 months, we subsampled two of the cores, but realized that dispensation from the exit port of the osmotic pump was

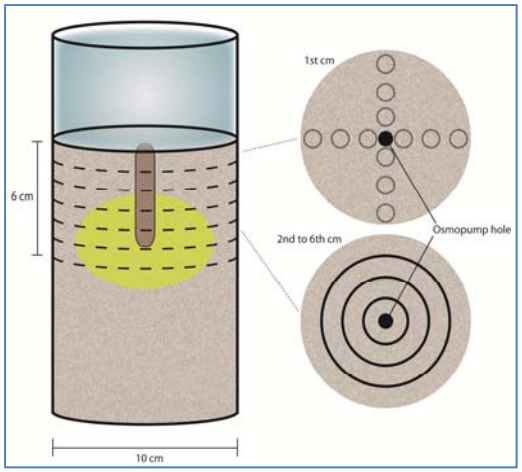

Figure 1. Schematic of sediment core and overlying water with osmotic pump in place (left) and of subsampling strategy (right). Yellow ellipse shows hypothetical range of calcein influence.

impeded. Thus, our "plan B" was to install new osmotic pumps using a different installation scheme (to prevent clogging). Our second attempt with benthic foraminifera began in late May 2013 after new cores were recovered. For this experiment, we placed the point source within 1 cm of the sediment-seawater interface. After ~4 months, a check of the cores indicates that benthic foraminifera did grow, incorporating the calcein (i.e., becoming fluorescent; Figure 2).

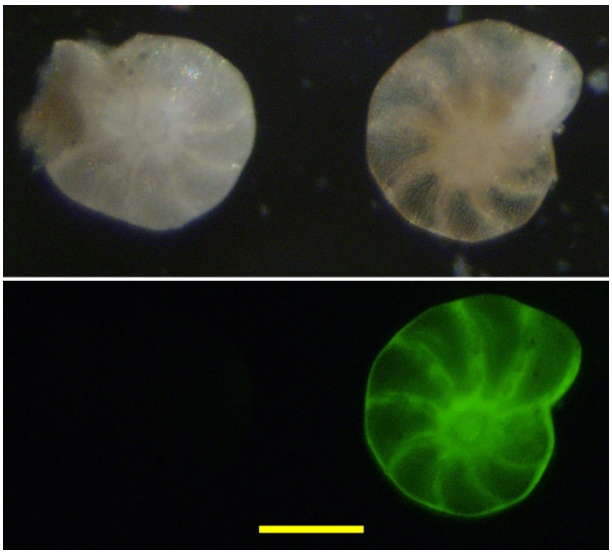

Figure 2. Paired images, reflected (top panel) and epifluorescence (bottom panel) of two *Elphidium excavatum* foraminifera. The specimen on the right calcified during the experiment because it is fluorescent (bottom right) contrary to the specimen on the left, which did not calcify during the experiment (no fluorescence in bottom panel). JMB and JCW, unpublished. Scale = 100 µm.

In addition, we tested the method on juvenile bivalves (surf clams and quahogs), starting in December 2012. Algal food was provided to each core every week. The surf clams did not survive long in the cores, but the quahogs remained active and grew. After ~4.5 months, the quahogs were removed from the cores. Specimens were preserved and examined with epifluorescence microscopy to determine if they had incorporated the fluorescent marker calcein. Many of the clams had portions of their shell that were brightly fluorescent (Figure 3). Clams grow somewhat like trees, where "rings" are added at any given time. Thus, in Figure 3, one can note the brightly fluorescent areas. When the clam moved away from the osmotic pump, the calcein did not become incorporated and thus was not fluorescent.

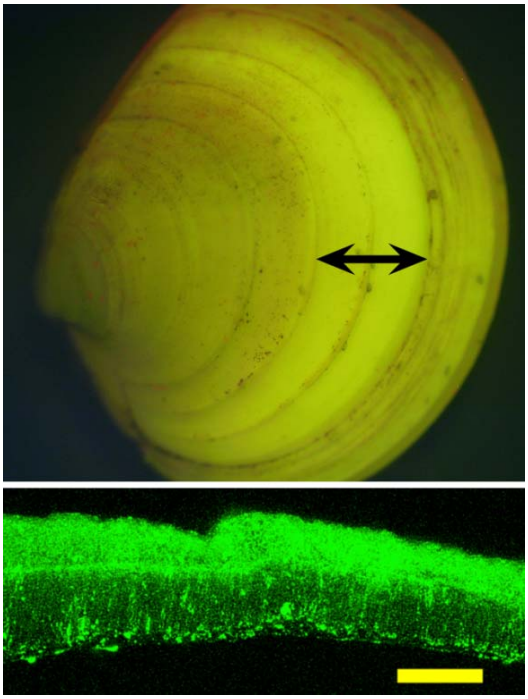

Figure 3 (view in color). Quahog *Mercenaria mercenaria*. Top: low magnification view of complete shell using epifluorescence microscopy. The brighter yellow zone delimited by the black double-ended arrow shows calcified section of shell during the experiment. The outer most section shows less intense fluorescence, suggesting the clam moved further from the osmotic pump point source. JMB and WP, unpubl. Scale bar = 200 µm.

While calcein has been used in both bivalve growth studies and foraminiferal studies, calcein has not been used as a point source in the environment. Pinpointing where and when similar organisms calcify is important because, for example, rates of shell growth in the deep sea are not well known. This osmopump method can be modified to deploy these units in the deep sea using a Remotely Operated Vehicle (e.g., *Jason*) or a Human Occupied Vehicle (e.g., *Alvin*). These units can be deployed in habitats that are spatially restricted (hydrocarbon seeps or other "extreme" environments), where we have little growth data for any sediment-dwelling species. These units can also, obviously, be deployed in shallow marine waters near shellfish fisheries and reefs with sediment pockets.

This project provided opportunities to a number of guest students and other researchers. More specifically, Francesco Mezzo, who was a WHOI Gori Fellow (Italian visiting student) was provided the opportunity to join the *Tioga* cruise. That was his first research cruise. William Phalen, a guest student from Boston College, helped perform the clam experiment along with setting up the most recent foraminiferal experiment. Megan Davis, a guest student from UNCW, joined the May 2013 cruise, helped collect the 2013 multicores, and assisted with set up and maintenance of the experiment during summer 2013. Two postdoctoral scholars (Anna McIntyre-Wressnig; Jos Wit) also assisted with the experiments.

A talk will be presented in late October 2013 at the Annual Meeting of the Geological Society of America (https://gsa.confex.com/gsa/2013AM/webprogram/Paper230776.html). This talk was invited by the session conveners. A short manuscript describing the method is being drafted and is expected to be submitted to a peer-reviewed journal by early 2014. Finally, a proposal will be submitted to NSF for additional funding to deploy this new tool in hydrocarbon-seep habitats, where it is important to establish the location and timing of calcification.