Ultrastructure of Hydrothermal Vent Foraminifers:
Novel in situ fixation approach to alleviate sampling artifacts

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FINAL REPORT

The goal of this Deep-Ocean Exploration Institute award was to investigate the cellular ultrastructure of benthic foraminifers such as *Abyssotherma pacifica* at the Galápagos vent field. In order to accomplish this goal, it was imperative that specimens be chemically fixed *in situ* so that possible sampling artifacts and trauma (from depth changes while alive) would not affect results. Thus, this project sought to address a fundamental cell biological question by utilizing novel sampling methods including *in situ* chemical fixation of foraminiferal-bearing hard substrates: Does *Abyssotherma pacifica* have prokaryotic symbionts or other cellular modifications to permit its inhabitation of hydrothermal vent areas? We developed a modified Enzymatic Sampler that was originally designed for *in situ* preservation of vent fauna with RNAlater, a preservative that allows for analysis of RNA and DNA. These modifications included larger chambers to accommodate 10x10 cm basalt colonization blocks for the collection of benthic foraminifera, a direct fluid / preservative delivery system that allows efficient manipulation of the preservative to sample ratio, and a fluid indicator that indicates when the sample has been fully treated with fixative.

The project was funded at <9% of our original request ($5,000 *vs*. $60,430). One sampler was constructed that contained four sampling chambers. Although DOEI funds were not provided for Bernhard's travel to join the cruise off the Galapagos, it was agreed that Bernhard would find other funds to join the cruise. Unfortunately, the *Atlantis/Alvin* schedule was delayed sufficiently that it was not possible for Bernhard to join that cruise as well as one of her own cruises, where she was Chief Scientist. Thus, Shank agreed to collect the proposed samples of agglutinated benthic foraminifers. Bernhard provided the proper chemicals for fixation from separate funds and also provided schematic and written descriptions of the endemic vent foraminifer *Abyssotherma pacifica*.

The Sampler was deployed successfully by *Alvin* in concert with the funded expedition to the Galápagos Rift in June 2005. Prior to the submersible entering the water, each of the preservation chambers was cleaned with ethanol. Four settlement blocks were recovered from a time-series experiment at the Marker B area in the Rosebud vent site on the final dive (#4124) to maximize the length of time for colonization (8 days). All four collection chambers were used for *in situ* injection of TEM-grade glutaraldehyde (3% final concentration) following the collection of basalts. Samplers injected the fixative into the sealed collection chamber slowly from the bottom to assist mixing and perfusion.

Following recovery, each of the 6 sides from each block were divided into cm grids and the distribution of microbial, faunal, and foraminifera were mapped photographically under a dissecting microscope, and placed in a separate vial for later examination. The total abundance of foraminifers per block was 12, 23, 24, and 9 on blocks 3, 4, 5, 6 respectively. Subsequent sample processing involved a transfer into cacodylate buffer (0.1 to 0.2 M sodium cacodylate; pH 7.6) and the maintenance at 4°C, including during transport to WHOI.

A total of nine samples were provided to Bernhard. Upon microscopic inspection at WHOI, it was noted that none of the samples had benthic foraminifers present. Photographs of the block surfaces indicate the foraminifers present were planktonic forms (and thus, of no interest because they are not endemic to the vent habitat). The lack of any identifiable foraminiferans in the samples indicates that were calcareous and the tests dissolved in the fixative/buffer.

We are currently preparing to conduct similar in-situ fixation collections of benthic foraminifera on the East Pacific Rise (with *Alvin*) in January 2007 (T. Shank, Chief Scientist) in which different post-preservation methods will be tested for successful collection. While the sampler has only been used on *Alvin* to date, it was specifically designed to be utilized by multiple submersible vehicles. The sampler currently resides at WHOI and is available for use.