Exploring Integrative Approaches to Understand the Interaction of Microbial Biofilms and Fluid Chemistry on Larval Settlement at Deep-sea Hydrothermal Vents

Timothy M. Shank, Stefan Sievert, and Stace Beaulieu

July 2003-June 2005 — Final project report

What were the primary questions you were trying to address with this research?

Biologists have long proposed that the chemical compositions of vent fluids serve as settlement cues for fauna to colonize vents along mid-ocean ridges; however, this hypothesis remains largely untested. In addition, microbial community composition in vent fluids and the development of microbial biofilms at vent openings would be expected to vary in response to vent fluid chemistry, and may facilitate or inhibit the settlement of invertebrate species. A major objective of our research is to understand biological/geochemical interactions during initial colonization of basalt at deep-sea hydrothermal vents, through time-series studies that combine molecular genetic characterization of colonists and *in situ* measurements of fluid chemistry. Understanding the factors that determine why larvae settle is a key first step to understanding the bigger picture of how thriving communities develop at hydrothermal vents.

What have you discovered or learned that you didn't know before you started this work?

- In < 2 weeks, microbial biofilms on the basalt panels had evidence of grazing by macrofauna such as limpets (Fig. 3).
- Macrofauna are capable of colonizing new basalt surfaces within just a few days (Fig. 5).
- Preliminary analyses of DNA extracted from basalt panels indicate changes in the microbial biofilm species composition in the first 2 weeks of deployment (Fig. 6).
- Gastropod limpets are capable of scoring basalt when actively feeding

What is the significance of your findings for others working in this field of inquiry and for the broader scientific community?

Our findings demonstrate how rapidly community development is initiated at hydrothermal vents in the deep sea. With further molecular analyses, we will gain insight into macrofauna-microbial associations that are established during the initial settlement of the basalt panels. Some of our original hypotheses were based on conclusions from biofouling studies of shallow-water marine invertebrates. Our study is important for the broader field of marine ecology to demonstrate the ubiquitous importance of microbial biofilms not only for potential cues to larval settlement, but also for the rapid development of diverse invertebrate communities.

What is the significance of this research for society?

Understanding the development of hydrothermal vent communities demonstrates the interplay of planetary renewal (geology), hydrothermal fluid chemistry, and microbiology in supporting higher life forms. These processes are fundamental to earth's life systems and may be on other planetary bodies.

What were the most unusual or unexpected results and opportunities in this investigation?

- Our most unusual finding was what appeared to be limpet feeding tracks on the panels deployed for 4- to 13-days at Experiment Site 1 within the tubeworm patch.
- The panel that was deployed for 13-days at Experiment Site 1 developed a thick, "fuzzy" biofilm of filamentous bacteria. In our follow-up study, which was conducted at a hydrothermal vent at the Galapagos Rift, a similar-looking "fuzzy" biofilm developed on panels deployed in a tubeworm patch for ~9-days.
- The initial 2-weeks of our experiment involved concurrent deployment of an in situ chemical sensor. When we recovered the sensor at the end of the cruise, adult worms (*Paralvinella grasslei*) had taken up extensive residence in the protective confines of the sensor head.

- We had originally proposed only a short-term deployment of the basalt panels, yet circumstances allowed us to leave several deployed for 2- and 9-months. These longer deployment periods gave us the opportunity to link and compare our results to previous colonization experiments at the EPR. In particular, the abundance of *Tevnia jerichonana* tubeworms after 9-mos in the *Riftia pachyptila* patch matched predictions from a published paper (Mullineaux et al. 2000).
- Another significant finding was that the temporal variability in fluid chemistry differed within a given vent assemblage (this has been dogmatically assumed for a long time, but not simultaneously measured in a vent system).

What were the greatest challenges and difficulties?

Our 1st challenge was designing the basalt panels. The panels had to be large enough to maximize counts of macrofaunal colonists, yet sections of each panel had to be small enough to extract the entire piece in minimal volume of DNA extraction buffer.
Our 2nd challenge was cutting the native basalt on a rock saw to construct the basalt panels.
Our 3rd challenge was on the seafloor… how best to recover panels that were hidden amongst tubeworms.
Our 4th challenge was in the lab… how to optimize the protocol for extracting DNA from the sections of the basalt panels.

When and where was this investigation conducted? (For instance, did you conduct new field research, or was this a new analysis of existing data?)

Our experiment was conducted at Tica Vent, a hydrothermal vent on the East Pacific Rise (9º 50' N, 104º 17' W). We deployed the basalt panels in Feb 2004 and collected some on the same cruise and the remainder on cruises in Apr and Nov 2004 (Fig. 1).

What were the key tools or instruments you used to conduct this research?

- Submersible Alvin
- Basalt panels (e.g. Fig. 4)
- Chemical sensor designed by Bill Seyfried and Kang Ding (UMinn)
- VEMCO temperature sensors (data in Fig. 2)
- In the lab: DGGE (BioRad) and DNA sequencer
- A new in-situ preservation sampler that fixes animals at depth for RNA expression studies

Is this research part of a larger project or program?

This project was a pilot study, and we conducted a comparative study at the Galapagos Rift in May 2005. This pilot effort has led to a larger 3-year National Science Foundation supported study on the East Pacific Rise that includes using our same approaches in broader ecological experiments.

What are your next steps?

- Complete analyses of microbial community composition
- Complete identification of macrofaunal colonists including DNA sequencing
- Write manuscripts detailing DOEI-funded study at EPR
- Compare results of DOEI-funded study at EPR to similar study at Galápagos Rift for more general conclusions about Eastern Pacific vent ecosystems
- Compare the genetic structure of captured larvae, juveniles (from panels), and adults to examine whether the recruits on the panels are part of cohort arriving from a distant vent location.

Have you published findings or web pages related to this research? Please provide a citation, reprint, and web link (when available).

Daily web log during cruise AT11-7 in Feb. 2004:
http://www.whoi.edu/atlantis117/feb17.html
DOEI Newsletter Oct 2004: http://www.whoi.edu/institutes/doei/general/print_news1004.html#3.
Oceanus Magazine July 2005: http://www.whoi.edu/oceanus/viewArticle.do?id=5367&sectionid=1000.

Figure panel

Fig. 1. Summary timeline for basalt panel deployments at Tica Vent on the East Pacific Rise.

Summary timeline for basalt panel deployments

Panel set	Total no. panels	Site: Expt. 1	Site: Expt. 2	Site: Ctrl.	Date (2004) Down	Date (2004) Up	Total no. days
Red 1	3	1	7	13	3 Feb	7 Feb	4
Red 2	3	17	11	5	7 Feb	16 Feb	9
White	3	3	9	15	3 Feb	16 Feb	13
Red 3	2	12	6		16 Feb	21 Apr	65
Green	3	2	8	14	3 Feb	19 Apr	76
Black	3	4		16	3 Feb	12 Nov 22 Nov	283 293

Fig. 2. Temperature sensors (ºC) attached to three basalt panels deployed in our experiment.

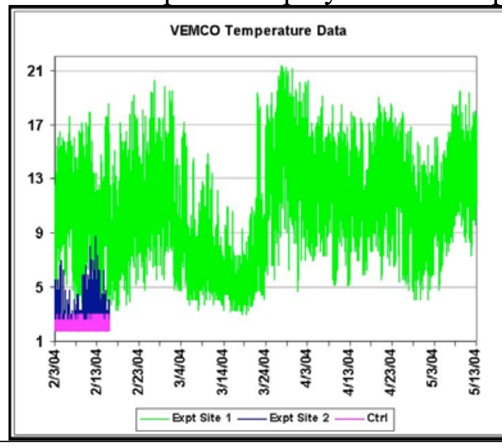


Fig. 3. Putative limpet feeding tracks on basalt panels deployed for 4-d at Expt Site 1.

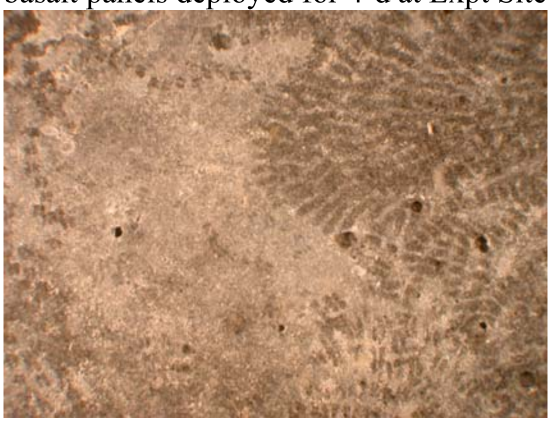


Fig. 4. Basalt panel with temperature sensor recovered after 9-mos, colonized by worms including *Tevnia jerichonana*, *Riftia pachyptila*, and *Paralvinella grasslei*.

Fig. 5. Macrofaunal colonists enumerated under a dissecting microscope.

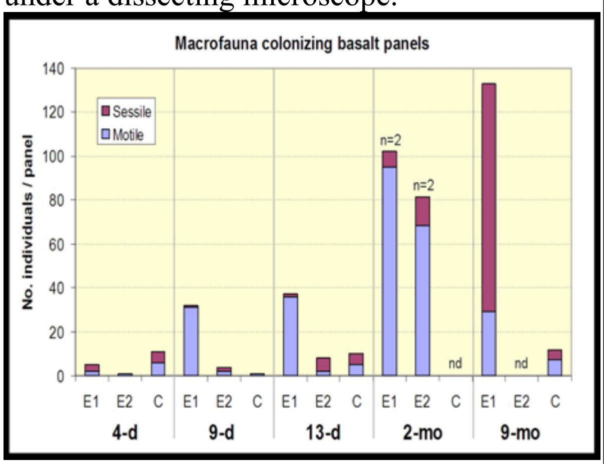
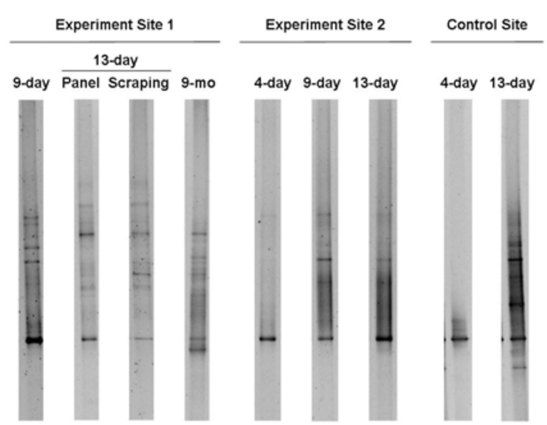


Fig. 6. DGGE gel banding pattern indicating differences in bacterial community composition among basalt panels.



Stace Beaulieu
Research Specialist

Biology Department
Woods Hole Oceanographic Institution

In 1998, I packed everything I owned into the back of my purple truck and drove across the country, from SIO in San Diego, to WHOI in Woods Hole for a postdoctoral scholarship. After 5 years in AOPE, I joined the Biology Department as a Research Specialist in 2003. I pretty much knew that I wanted to study deep-sea biology since I was about 7 years old and saw photos of hydrothermal vents in National Geographic. At about that time, growing up in Florida, my best friend's father used to take us snorkeling while he scuba'd for lobsters, and every so often we would breathe from his scuba regulator underwater. Back in high school, I was quoted as saying that I didn't know whether to become a marine biologist or an ocean engineer. Luckily, research in deep-sea biology enables me to combine skills from both fields. In addition to research at WHOI, I enjoy teaching the JP courses in biological oceanography and marine invertebrates. Outside of WHOI, I am most active in the Falmouth Track Club and with the Town of Falmouth Bikeway Committee. I met my husband, Steve Faluotico (AOPE), because we went for a run before boarding Atlantis for a cruise to the EPR in 2000.

Dr. Timothy M. Shank
Associate Scientist
Biology Department
Woods Hole Oceanographic Institution

Tim Shank earned a bachelor's degree in biology and German from the University of North Carolina at Chapel Hill. After college he worked in the Genetic Toxicology Division of the Environmental Protection Agency to investigate how airborne pollution creates fine-scale genetic damage, the precursor to cancer growth. Applying genetic approaches developed during this research, he received a Ph.D. in ecology and evolution from Rutgers University, where he examined the evolution of deep-sea hydrothermal vent communities and the genetic relationships of vent fauna and their adaptations to thrive in extreme chemosynthetic environments. His research interests focus on understanding the evolution of life in the deep sea and the ecological processes responsible for creating and maintaining biodiversity in the oceans. He combines molecular genetic approaches and ecological field studies to understand the conditions and adaptations that allow various species to migrate, evolve, and thrive along the global mid-ocean ridge system. His studies include a new class of deep-sea hydrothermal system, discovered in 2001, where fauna have evolved in conditions similar to pre-biotic earth. He has co-discovered more than ten hydrothermal vent systems in the Atlantic, Indian, eastern and western Pacific Oceans. He has authored or co-authored more than 50 scientific research publications and conducted more than 25 scientific expeditions to deep-sea hydrothermal vents, hydrocarbon seeps, continental slopes, and seamounts throughout the world's oceans, including the North Atlantic, the Sea of Cortez, the central Indian Ocean, and eastern, northeast, and southeast Pacific. He has participated in more than 50 submersible dives, 30 remotely operated vehicle dives, and 35 autonomous underwater vehicle dives, and soon, will take part in the first use of a full-ocean depth hybrid remotely operated vehicle capable of diving to the deepest regions of the oceans to explore the evolution of life under most extreme pressure conditions. Shank has been involved in many educational and public outreach activities that have reached millions of individuals. He developed a high school and undergraduate educational CD on hydrothermal vents, as well as Web-based teaching modules for the NOAA OceanExplorer.gov and DiveandDiscover.com Web portals. His most recent award, the NOAA OceanAGE award, is the latest vehicle through which Shank interacts with students throughout the world to convey firsthand knowledge of careers in marine science through live Web interviews, profiles, and mission logs. His research efforts have been featured in *Science Discover* and other magazines; on National Public Radio, and on numerous nationally broadcast television documentaries on The Discovery Channel, BBC, The National Geographic channel.