Characterization of vent-associated protist communities

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In the decades since the discovery of hydrothermal vents biological investigations in these environments have been focused on either chemosynthetic prokaryotes (bacteria and archaea) or multicellular eukaryotes (macrofauna). Only recently have we begun to think about the unicellular eukaryotes (i.e. protists) that inhabit these environments. What role do they play in ecosystem functioning? How do they cope with the toxicity of the environment? How does the protozoan population respond to changing conditions in dynamic hydrothermal settings? What is the range of various protozoan groups with respect to the source of hydrothermal venting? Are there endemic hydrothermal populations of protists?

My participation in the 2014 Ironman/Submarine Ring of Fire cruise aimed to delve into all of these questions by assessing 18S rRNA gene diversity of bulk community DNA and RNA, isolating previously identified key organisms for single cell genome sequencing, quantifying the abundance of previously identified key groups in various vent and non-vent environments, and attempting to culture previously uncultured protist groups that were dominant in past samples from Mariana vents. This work was to build upon molecular analyses of samples from the 2004 and 2006 Submarine Ring of Fire cruises thus creating a time-series component to the study. The primary objectives were 1) time-series sampling at key vents on NW Rota-1 and NW Eifuku, 2) niche comparisons of protozoan communities (diffuse fluids, plume, mat, background seawater), and 3) sampling for cell sorting and single-cell genomics. Secondary objectives included 1) microscopic observations of protists from vent fluids, 2) collection of animals for parasite investigations, and 3) testing methods for culturing of vent protists.

Due to the series of unfortunate weather and equipment delays the sampling of vent fluids was not carried out in a manner that will be of much use for comparison to previous sampling years, thus the time-series aspect was not achieved. Additionally, the current state of NW Rota-1, which has significantly quieted down from previous samplings, left little opportunity for such time-series investigations in that location. DNA samples from weakly venting fluids with temperatures barely above ambient, and from smoky plumes in the area may provide an interesting opportunity to monitor changes in the protist community in the waters surrounding the now quiet volcano. Plume samples were also collected over the summits of Daikoku, which is now showing signs of intense eruptive activity, and over NW Eifuku. These three plume samples will be valuable in assessing whether subsurface microbes expelled with hydrothermal fluids act as an attractive food source luring in protist predators and linking the subsurface and deep-ocean food webs.

Subsamples of iron mats collected by the Moyer and Emerson groups were preserved for CARD-FISH, a microscopic probing technique that will allow visualization and enumeration of protist groups that have been previously identified as dominant members at the Mariana vents. Microbial mats may provide and excellent source of food and/or shelter for protists but this association has not yet been investigated. Samples were also preserved for CARD-FISH from tissues of shrimp, mussels, scale worms and limpet larvae to test the theory that dense communities of animals serve as habitats for parasitic protists in the hydrothermal vent environment.

By far, the most successful and surprising aspect of protist research on the cruise came from the culturing attempts. While this was not a primary objective and thought to be simply a first pass attempt, the results were very encouraging. Positive cultures resulted from inoculation with diffuse fluids, plume water, and water from within microbial mat sampling devices. Cultures of recently hatched limpet larvae also produced biofilms that appeared to be comprised of protists. Three colonization devices borrowed from Pete Countway (Bigelow) were deployed for five days at NW Eifuku adjacent to 17-34°C diffuse fluids. Material from these devices also yielded positive cultures of what appear to be a fairly uncharacterized group of eukaryotes.

Samples of diffuse fluids and mats were collected for DNA from the Urashima vent area, which has not previously been investigated for protist diversity. This site is in the south near the junction of the volcanic arc and back arc and will add a new dimension to previous arc-scale samplings. Iron mats from this site will be used to test how the addition of iron influences the growth of protists in culture.