Characterizing Population Dynamics of Planktonic Archaea in the North Pacific Subtropical Gyre: 
A C-MORE Edventures Proposal

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Project Summary:

Intellectual Merit: Planktonic microorganisms belonging to the domain Archaea are abundant and ubiquitous in ocean ecosystems. However, despite comprising a major fraction of living biomass in the sea the ecological and biogeochemical roles these organisms play are largely unknown. We propose to investigate temporal and spatial variability in the population dynamics of Archaea in the oligotrophic North Pacific Subtropical Gyre (NPSG) to shed insight into three broad questions: 1) What controls the vertical distribution of Crenarchaea in the sea? 2) What limits archaeal population sizes and diversity in the upper ocean? 3) Are there robust and predictable temporal dynamics associated with different archaeal populations in the NPSG? We propose to address these questions through a combination of laboratory analyses and field-based experiments. Field sampling and experiments will be based at Station ALOHA (22.75N, 158W), the field outpost for the Hawaii Ocean Time-series (HOT) program, thereby providing a robust time context for our observations. In addition, several experiments aimed at evaluating controls on archaeal population dynamics will be conducted as part of a funded 10 day cruise (August 20-30, 2010) in the NPSG. Finally, nucleic acid samples collected over the past 4 years at Station ALOHA on the near-monthly HOT cruises will be analyzed to gain information on time and space (vertical) dynamics associated with Archaea in this ecosystem. By merging information gained from time-series sample analyses and field experiments, we seek insight into specific processes controlling fluctuations in population size and diversity of planktonic Archaea in the sea.

Broader Impacts: The proposed work will be conducted by Brenner Wai, with day-to-day mentoring from C-MORE post-doc Daniela Böttjer and C-MORE investigator Matthew Church. Brenner has worked with Church over the past three years through the C-MORE scholar program; as a student of native Hawaiian ancestry, his involvement in the proposed work provides direct and relevant ties to a community of people currently underrepresented among STEM career paths. The project seeks salary support for Brenner to continue his involvement in C-MORE following his successful graduation from the Global Environmental Sciences program at the University Hawaii. Brenner intends to apply to the Department of Oceanography at the University of Hawaii for graduate studies in the Spring 2011. The proposed work builds heavily on Brenner’s previous successes as a C-MORE scholar, and creates new opportunities for him to continue his education and research training in microbial oceanography. As such, this proposal directly addresses two major C-MORE education goals: A) producing leaders in the next generation of microbial oceanographers by providing state-of-the-art training, and B) increase the number of underrepresented groups in STEM career paths.

Introduction and Background: Planktonic Archaea are among the most abundant cellular life forms in the sea comprising upwards of 10^{28} cells (Karner et al. 2001). Although historically considered as extemophiles, Archaea are now known to inhabit diverse environments including a wide variety of aquatic habitats (DeLong 2007). In recent years, the combination of geochemical and molecular biology-based approaches has provided new insights into the potential significance of these organisms to elemental cycling in the sea. In particular, gene-based surveys led to the understanding that marine Archaea generally fall into two major population lineages: the Crenarchaea, whose notable cultivated members include the extreme thermophiles; and the Euryarcheota, which include the halophiles and methanogens (DeLong 2007). Early application of techniques such as fluorescence in situ hybridization
(FISH) revealed that members of the Crenarchaeota are dominant picoplankton in the sea (DeLong 1992, Karner et al. 2001, Church et al. 2003); these microbes often appear in low abundances (<10^2 cells L^-1; accounting for ~1-5% of the total picoplankton abundances) in the well-lit upper ocean waters, but increase several orders of magnitude (upwards of 10^5 cells L^-1) with depth into the meso- and bathypelagic waters, making them the dominant group of picoplankton in the deep sea (Karner et al. 2001). In contrast, members of the Euryarchaeota are often most abundant in the upper ocean waters (Karner et al. 2001, Mincer et al. 2007). To date, it remains unclear what processes dictate the vertical segregation of these assemblages, and we have only limited understanding of the roles these organisms play in plankton ecology or biogeochemical cycling. Insight into probable energy and reductant sources supporting archaeal growth has stemmed from metagenomic surveys identifying genes encoding the ammonia monooxygenase protein in members of the Crenarchaeota (Venter et al. 2004, Treusch et al. 2005). This protein catalyzes ammonia oxidation (Rotthauwe et al. 1997), the initial step in nitrification, a major component of the nitrogen cycle. Since the initial discovery, analyses of amoA genes (encoding subunit A of the ammonia monooxygenase protein) and amoA gene transcripts have provided a useful tool for studying the diversity (Francis et al. 2005) distributions (Mincer et al. 2007, Agogue et al. 2008, Beman et al. 2008), and transcriptional activities (Frias-Lopez et al. 2008, Church et al. 2010) of nitrifying Crenarchaeota in the sea. However there is little information on variability (space or time) in population dynamics associated with the Euryarchaeota.

Our previous research has provided insight into several important time and space dynamics associated with planktonic Archaea: 1) despite low abundances, Crenarchaeota appear most transcriptionally most active in ammonia oxidation in the upper ocean waters (Church et al. 2010); 2) physical perturbations, such as those associated with mixing or mesoscale upwelling, act to distribute Crenarchaeota into the upper ocean, but following such perturbations abundances of these organisms rapidly declines (Wai and Church, unpublished data); and 3) mesopelagic inventories of Crenarchaeota demonstrate a moderate seasonal pattern, increasing in the late summer and fall coincident with periods of elevated particulate nitrogen flux to the deep sea. The proposed work seeks mechanistic understanding of the role that various biotic and abiotic controls play in determining the spatial and temporal dynamics in archaeal populations in the sea. Most notably, we will employ at sea experiments to examine whether sunlight plays an important role in determining the vertical structure and transcriptional activities of these microorganisms. In addition, we will examine the relative competitive successes of nitrifying Archaea and Bacteria to varying concentrations of ammonia (Erguder et al. 2009, Martens-Habbena et al. 2009). Finally, we will continue to evaluate temporal and spatial dynamics in archaeal population structure, including Euryarchaeota, from an existing nucleic acid sample time series archive collected at Station ALOHA.

**Specific Research Approach and Objectives:** With support from the C-MORE scholars program, Brenner Wai has overseen the analyses of archaeal population dynamics for the Church lab. To date, this effort has included extraction of nucleic acids from the C-MORE BULA transect and monthly HOT program cruises; quantitative PCR (QPCR) and reverse transcriptase QPCR (RT-QPCR) of crenarchaeal amoA and 16S rRNA genes and amoA gene transcripts from the CMORE BULA cruise; and analyses of crenarchaeal amoA gene abundances from HOT cruise samples (2005-2009). Over the next several months (beginning in July 2010), we seek support for Brenner (presumably hired as a casual hire UH employee) to continue his laboratory analyses to include RT-QPCR based determination of temporal variability associated with amoA gene transcripts at Station ALOHA; QPCR analyses of Euryarchaeota gene abundances (based on 16S rRNA gene amplification), and examination of vertical and temporal
In addition to continuation of these laboratory analyses, Brenner would oversee field-based experiments to evaluate specific controls on the vertical distributions and population dynamics associated with both Crenarchaea and Euryarchaea in the NPSG. This would entail participating in 4 HOT cruises (July, August, and September 2010) and a 10 day cruise (August 20-30, 2010) in the NPSG in support of an NSF funded project on nitrogen fixation (expedition led by Church). While at sea, whole seawater will be collected from near the base of the euphotic zone (125 m) where crenarchaeal abundances increase sharply, amended with various concentrations of ammonia (10 nM to 1 µM), and incubated aboard the ship under different light shading (ranging from dark to 20% surface irradiance) for 4-10 days. Subsamples will be collected every 12 hours for determination of archaeal abundances (by QPCR) and amoA transcriptional activities (by RT-QPCR), and determinations of the abundances of Euryarchaea and bacterial nitrifiers (by QPCR). Dr. Böttjer will be aboard these cruises to provide intellectual guidance and assist in the experimental design and sampling.

**Mentorship and Outreach:** Since joining Church’s research group in 2008, Brenner has become a productive contributor to the lab; moreover, he has gained valuable skills allowing him to become increasingly independent in his work. For the proposed project, Brenner will continue to receive direct scientific mentorship from Church; however, in an effort to develop an organized peer-mentoring structure for the lab, post-doc Böttjer will begin to oversee Brenner’s day to day activities, notably including his work aboard research cruises. This will provide Böttjer with valuable experience in mentoring a research project, while also providing an immediate point of contact to supervise Brenner’s work. Brenner would keep regular work hours, with the expectation that he would contribute 40 hours of work a week. In addition to his laboratory responsibilities and at sea research, Brenner would continue to be available to contribute to the outreach and educational programs through the CMORE Education office.

**Deliverables:** Results from the proposed project will be written for peer-reviewed publications. Brenner will be involved in all stages of this publication, including providing and analyzing data, and contributing to the drafting of manuscripts. We anticipate support from this project contributing to at least 2 publications; one describing temporal variability in archaeal population dynamics (based on analyses of existing HOT cruise samples) and another publication describing the results of the incubation experiments. In addition, Brenner would present a poster describing the proposed work at the annual CMORE All Hands meeting in 2011 and at the 2011 American Society of Limnology and Oceanography (ASLO) meeting in San Juan, Puerto Rico (February 13-18, 2011). A final report (including photos) summarizing the outcomes and activities of the proposed work would be submitted to the CMORE Education office.

**Budget Justification:** We have requested 14 weeks of salary support for Brenner Wai for work between July 5 and October 8, 2010. The requested level of support (totaling $9,596) assumes Brenner would be hired as a stipend employee, inclusive of fringe (2.3%). Church will utilize his annual CMORE supply budget allocation to support costs associated with the project, including consumables required for lab work (DNA/RNA extraction kits, PCR reagents, enzymes, plasticware, etc.) and any supplies needed for the at-sea sample collections. We have also requested $2,800 for Brenner to attend and present the results of his research at the 2011 ASLO meeting in San Juan, Puerto Rico. This includes $1,100 for airfare (R/T Honolulu to San Juan, Puerto Rico), $600 for hotel, $600 for meals, and $400 for the meeting registration.
Itemized Budget:

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References