Searching for microbes in deep-sea seep and hydrothermal vents using the Environmental Sample Processor.
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Introduction: In the deep sea, organisms must contend with a variety of unique environmental challenges. Darkness, high pressures, hot or cold temperatures, and a conductive and corrosive liquid environment have resulted in unique life-forms found no where else on earth. Instrument systems designed to study these creatures in situ and characterize the biogeochemical transformations they mediate must also contend with the same environmental challenges, particularly if deployed for extened periods. We have been addressing this problem by developing an instrument package for autonomously sampling and detecting microbes found in Earth's deep-sea seep and hydrothermal vent fluids. Our objectives are to advance our understanding how life thrives in extreme ennvronments from a remote sensory context as well to provide model sytems for developing the scientific and technical capability for searching for life on other planets. To accomplish these goals we are modifying an experimental oceanographic instrument known as the Environmental Sample Processor (ESP; http://www.mbari.org/esp) to work at depths from 200m to 4000m. We refer to this instrument as the deep-sea ESP (D-ESP; Fig. 1.).

The D-ESP is a device that allows for autonomous application of molecular probe assays and qPCR in surface and deep-water ocean environments to provide near real-time detection of target molecules. The instrument also allows for preservation of samples so that material can be returned to a laboratory for further analyses. The system consists of three major components: an external sampling module, a core sample processor ("core ESP"), and analytical modules [1]. These components allow for a cascade of autonomous operations from acquiring a coarse-screened (~0.5mm pore size mesh), multi-liter sample, to the detection and quantification of specific nucleic acid sequences or other biotic materials on a microfluidic scale. The D-ESP is bundled with chemical and physical sensors ("contextual sensors") so that samples collected, and the assay results obtained, can be referenced against the concurrent environmental background. Currently, near real-time observations are achieved using low density DNA probe and protein arrays, and qPCR. Filter-based sandwich hybridization methodology enables direct detection of ribosomal RNA sequences indicative of groups of Bacteria and Archaea, as well as a variety of invertebrates and harmful algal species.. The ESP has been fielded on a number of platforms including moorings, piers, ROVs and benthic "elevators" for periods in excess of 30days, and operated as a standalone system or in concert with other networked ocean observatory infrastructure.

Fig. 1. The D-ESP being readied for deployment to 900m.

Operations: The core ESP concentrates particultates from large volumes of seawater (liters) using direct flow filtration, with DNA and protein array assays following on a millifluidic scale. However, many detection chemistries require precise manipulation and analysis of microfluidic sample volumes. To meet that requirement we designed a separate fluid handling station that can be added to the existing core ESP. This device is known as the "microfluidic block", or MFB. The MFB distributes, on a microfluidic scale, samples and reagents to one or more detection modules. The current detection module supports a reusable solid phase extraction column for purifying nucleic acids and a 2-channel real-time PCR device. The MFB supports the use of a variety of PCR master mixes, primer/probe combinations and control templates. As a first step toward working at depth, the ESP has per-

performed sample collection, processing, and analysis in surface waters. Preliminary results show congruence between the DNA probe arrays targeting rRNA and qPCR methodologies targeting rDNA (Fig. 2).

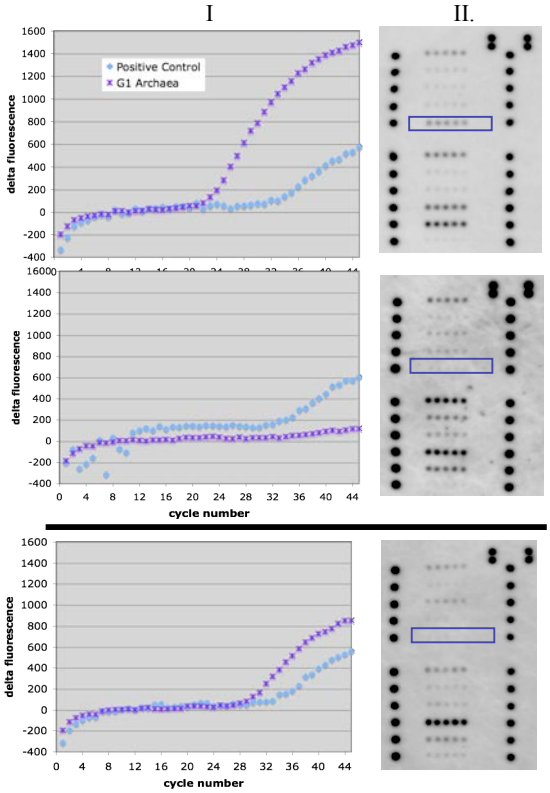

Fig. 2. Assay results from 3 separate samples collected during a 1-month surface deployment. PCR results (Col. I) and DNA probe arrays targeting rRNA highlighting detection of G1 Archaea (identified in boxes, Col. II). Both PCR and priobe arrays identify G1 in row 1, and both show its absence in row 2. Row 3: PCR detects G1 but SHA does not. Blue symbols are a positive control showing that amplification occurred [2]

A longer time series collected in situ reveals relationships between the abundance of microbes belonging to the ubiquitous G1 Archaea clade and prevailing environmental conditions (Fig. 3).

The MFB, with its attendant detection devices, can be added to or removed from the core ESP as a single unit with minimal disassembly. This allows for distribution of the MFB to multiple laboratories for testing, configuration, and protocol development/validation. A number of research groups are now adopting the ESP/MFB as a platform for fielding new analytical modules including surface plasmon resonance and capillary waveguides. The MFB and new analytical modules offer opportunities for reducing the overall size and power requirements of the ESP, such that it could be designed for use on interplanetary missions.

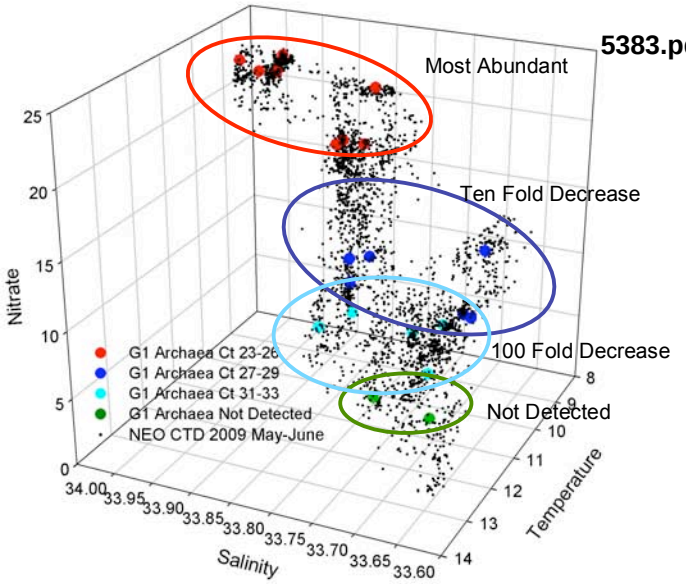

Fig. 3. Environmental data (black dots; x, y, and z axes) from a 1 month surface deployment with the ESP running qPCR. Larger colored dots show abundance of G1 Archaea based on C_t values during PCR. Ovals show rough groupings. G1 Archaea abundance is strongly correlated with nitrate concentration ($R^2 = 0.79$). From Preston et al., unpubl.

Near Future: Funding provided by NASA's ASTEP program continues to allow the fabrication and installation of an *in situ* mass spectrometer (ISMS) for detection and quantification of volatiles up to 300 amu and an *in situ* underwater spectrometer (ISUS) for assessing concentrations of nitrate, hydrogen sulfide, and various aromatic compounds. Our objective is to characterize time-dependent changes in microbial community structure and gene expression as a function of variable chemical/physical environmental conditions. We also aim to develop an adapative sampling response that is triggered by environmental assessments provided by onboard contextual sensors as well as other elements of an ocean observatory network.

Discussion: Here we review the technical challenges of operating the D-ESP at depths >1000m, and science results from two ~1 month D-ESP deployments on the Monterey Bay Accelerated Research System (MARS; http://www.mbari.org/mars/). These deployments, are in preparation for upcoming field campaigns planned to methane seeps in July 2010, as well as methane hydrate outcroppings and hydrothermal vents along the U.S. west coast (2011). Finally, we outline opportunities this platform provides to astroibiologists who seek a testbed to prepare and trial instrumentation for operations in harsh environment as a step towards flight qualification.

References:

[1] Scholin C., et al. (2009) *Oceanography 22,* 158-167

[2] Preston C., et al. (2009) *Env. Microbiol 11*, 1168-1180