Deep Water Instrument for Microbial Identification, Quantification, and Archiving

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ABSTRACT

Here we report on a robotic system known as the Environmental Sample Processor (ESP) that conducts molecular biological analyses *in situ* as a means of identifying and quantifying a variety of microscopic organisms at oceanic depths up to 4000m. The ESP concentrates microbes through filtration and then detects these microbes using low-density DNA probe arrays, protein arrays [1-3], and real-time quantitative PCR [4, 5]. Samples can also be preserved for further analysis after instrument recovery [6]. The deployment platform also carries external chemical and physical sensors to provide contextual measurements of the surrounding environment.

Because the ESP performs all collection and analytical functions within a one-atmosphere pressure housing using fluidic equipment rated for only 500kPa, several technical developments were necessary to acquire high pressure ambient water samples for processing by the core instrument. This 4000m depth-hardened version of the ESP is referred to as the Deep-Sea ESP, or D-ESP.

The D-ESP platform was designed to be easy to deploy, recover, manipulate while on the seafloor, and operate on batteries if necessary. In addition, the sample intake system can target specific areas nearby on the seafloor, safely collecting heated water from diffuse hydrothermal vents.

Here we report preliminary data from the deployment of D-ESP into the caldera of an active underwater volcano off the Oregon coast from July 15-20, 2011. To accomplish this, a number of items required special engineering consideration:

Pressure Housing

The D-ESP and support electronics required a 4000m rated spherical titanium housing over 1m in diameter, machined from near-net-shape castings.

In Situ Pressure Reduction

We developed a pressure-modifying interface called the Deep Water Sampling Module (DWSM) to collect discrete samples of seawater and provide them to the instrument at greatly reduced pressure, allowing the ESP to process the sample normally.

Benthic Lander

When deployed, the D-ESP system is within a free-fall benthic lander, about 2.5m tall and 2m in diameter (Fig. 1). This assembly contains the ESP and DWSM, an In Situ Ultraviolet Spectrophotometer (ISUS), an In Situ Mass Spectrometer (ISMS), a CTD, and other contextual sensors. It also carries an acoustic communication modem, tracking beacons, batteries, buoyancy foam, and a drop weight with release system. Once deployed, the benthic lander can be repositioned by an ROV.

Fig. 1. D-ESP lander just before deployment. Titanium sphere houses the ESP instrument. In this view, DWSM is on the opposite side of the sphere.

Sampling Wand

A bifurcated sampling wand system was developed to allow targeted water collection from cracks, seeps, or vents. In the event that sample water was at elevated temperature, the wand acted as a heat exchanger, cooling the water to near ambient before it entered the DWSM, ESP, and contextual sensors. Additionally, a temperature sensor located at the wand tip monitored and recorded vent temperature, preventing sampling if outside acceptable limits. This system could select from two different sampling locations, the wand end (e.g., at the vent) or the ambient seawater around the lander, without ROV servicing to reposition the instrument or wand.

Battery Sleds

The D-ESP was designed to operate continuously when powered from a submarine cable or for short time periods with onboard batteries. Because of substantial power demands (170W avg., 600W peak) and the lack of submarine cable power at the 2011 deployment site, we developed *in situ* charging processes and auxiliary battery sleds to augment the onboard battery power. This extended the ESP operating time and maintained power to all systems in the event that the ship/ROV needed to be recalled for extended periods. The battery sleds were deployed, swapped and recovered with the ROV (Fig. 2).

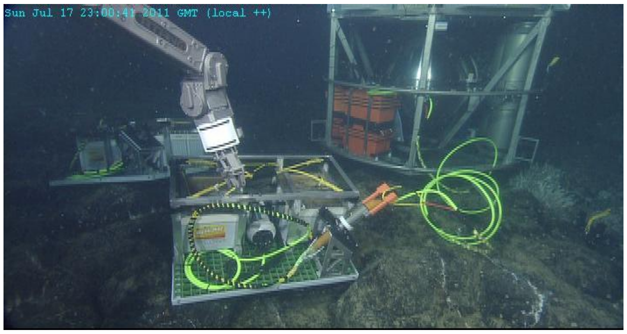

Fig. 2. Deep ESP deployed at the ASHES vent field within the Axial seamount caldera. An auxiliary battery pack is being repositioned by ROV.

Axial Seamount Deployment

Axial Seamount is an active underwater volcano ~470km west of the Oregon Coast with a recorded history of eruptions, the most recent being in 2011. We deployed the D-ESP in a diffuse vent field known as ASHES (Axial Seamount Hydrothermal Emissions Study) to determine if microbial populations being ejected from vents could be distinguished from those living in the surrounding benthic water autonomously, in real-time. The wand system permitted uninterrupted sampling at both the vent and at the lander, ~10m away. Contextual sensors on both the wand and at the lander showed significant difference in water properties, and microbial analyses conducted *in situ* clearly revealed different populations inhabiting vent vs. non-vent waters (Fig. 3).

The D-ESP is currently deployed and operating on the Monterey Accelerated Research System (MARS) in Monterey Bay.[8]

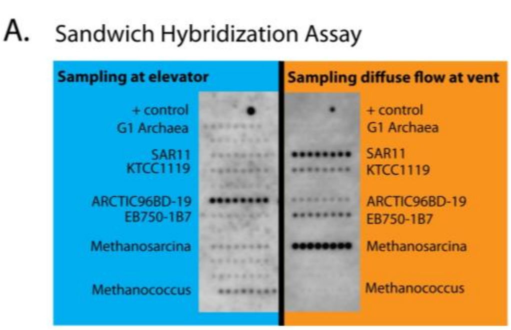

Fig. 3. Data obtained while operating the D-ESP at ASHES. (A) Sandwich hybridization shows differences in rRNA community structure when sampling away from or within the vent.

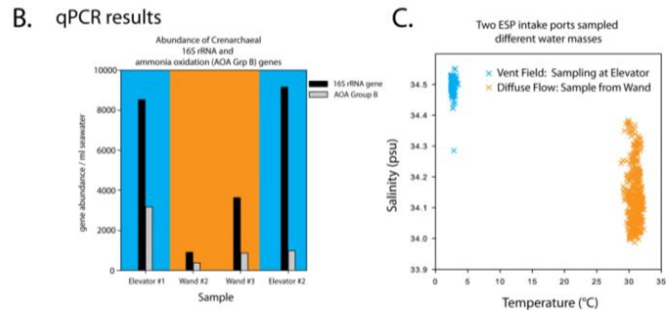

Fig. 3 (cont). (B) Real-time qPCR shows differences in gene copy number (Group 1A and AOAb genes) depending on collection site; (C) temperature/salinity plot shows samples collected are from two distinct sources.

References

[1] Scholin, C, G. Doucette, S. Jensen, B. Roman, D. Pargett, R. Marin III, C. Preston, W. Jones, J. Feldman, C. Everlove, A. Harris, N. Alvarado, E. Massion, J. Birch, D. Greenfield, R. Vrijenhoek, C. Mikulski, K. Jones. “Remote detection of marine microbes, small invertebrates, harmful algae and biotoxins using the Environmental Sample Processor (ESP)” 2009, *Oceanography* 22:158-167.

[2] Environmental Sampling Processor website: http://www.mbari.org/esp/

[3] Greenfield, D., R. Marin III, G.J. Doucette, C. Mikulski, S. Jensen, B. Roman, N. Alvarado, C.A. Scholin. “Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007.” 2008, *Limnology and Oceanography: Methods* 6: 667-679.

[4] Preston, C., A. Harris, J. Ryan, B. Roman, R. Marin III, S. Jensen, C. Everlove, J. Birch, J. Dzenitis, D. Pargett, M. Adachi, K. Turk, J. Zehr, C. Scholin. “Underwater application of quantitative PCR on an ocean mooring.” 2011, *PLoS ONE* 6(8): e22522. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0022522

[5] Robidart JC, Preston CM, Paerl RW, Turk KA, Mosier AC, Francis CA, Scholin CA, Zehr JP. Seasonal Synechococcus and Thaumarchaeal population dynamics examined with high resolution with remote in situ instrumentation. ISME J. 2012 Mar;6(3):513-23

[6] Ottesen E, Robidart J, Preston, C. Scholin C., and E.F. DeLong 2011. “Metatranscriptomic analysis of autonomously collected and preserved marine bacterioplankton.” 2011, *ISME Journal* epub: http://www.nature.com/ismej/journal/vaop/ncurrent/full/ismej201170a.html

[7] Roman, B., C. Scholin, S. Jensen, E. Massion, R, Marin III, C. Preston, D. Greenfield, W. Jones, and K. Wheeler, “Controlling a Robotic Marine Water Sampler with the Ruby Scripting Language” *Journal of the Association for Laboratory Automation*, Volume 12, Issue 1, February 2007, Pages 56-61

[8] Massion, E. and K. Raybould (2006). MARS, the Monterey Accelerated Research System. *Sea Technology*, 47: 39-42.