Project Title: Deep-sea Environmental Sample Processor

Sanctuary: Monterey Bay National Marine Sanctuary

Project Coordinator:
Dr. Chris Scholin, MBARI Research Chair
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Project Abstract: The application of molecular analytical techniques is pervasive in the ocean sciences, yet the vast majority of work typically occurs in a shore-based laboratory after return of a discrete set of samples collected at sea. The overall goal of this project has been to develop an instrument known as the Environmental Sample Processor (ESP) to overcome that limitation (http://www.mbari.org/microbial/esp/). The ESP allows for application of molecular probe technology *in situ* to enable remote detection of water-borne microorganisms, their genes and gene products. A long-term goal is to create the ability to deploy an array of internet-accessible ESPs in support of basic environmental research and resource management activities such as those outlined by the OOI/ORION (http://www.orionprogram.org/OOI/default.html), IOOS (http://opendap.co-ops.nos.noaa.gov/content/), and COHH (http://www.whoi.edu/science/cohh/index.htm) (etc.) initiatives. Here, the primary goal is to explore application of the ESP in the deep-sea using the MARS cable node as a test bed. (Alternate versions of the ESP have been routinely deployed in the Sanctuary in shallower water.)

Project Rationale: Cabled observatories offer many opportunities for developing and applying novel technologies for the study and monitoring of the deep-sea. The MARS node was established as a test bed to help realize that vision. Deploying the D-ESP on MARS will help us to refine marine operation procedures associated with deploying, operating, and recovering custom instrumentation in the deep ocean. It will also serve to validate its performance ahead of deployments elsewhere in the world.

Objectives: The three main objectives of this project are:

1. Deploy D-ESP on the MARS node.
2. Operate the instrument for several months.
3. Use the D-ESP to detect marine microbes and invertebrate larvae in time series fashion.

Please see http://www.mbari.org/mars/general/deep_esp.html for more information on the purpose and benefits of deploying the D-ESP on MARS. A printed copy of this webpage is included in this packet.

Intended Use of Results: Scientific and engineering publications and presentations.

Procedures: The ESP instrument (see Figure 1 below) and attendant contextual sensors are deployed on the seafloor using an "elevator" (common procedure). The instrument package will be picked up using an ROV and placed at an experimental site near the MARS node. The ROV will connect the D-ESP to the MARS node. Once on the cable, the science and engineering team will operate the water sample collection and molecular analytical components of the instrument.

Collections: The ESP will be used to collect (via membrane filtration) particulate material that is smaller than ~1mm and greater than ~0.2 µm. The material collected is infused with saline ethanol to preserve samples for microcopy and DNA analysis. Samples of microbes from this area/depth are not unique. What is collected will be archived at MBARI and made available to the research community.

Schedule: Currently, the plan is to deploy the D-ESP on MARS approximately the fourth quarter 2008 and recover in the first quarter 2009.

Publications and Reports: Scientific papers and reports, journals TBD.

Data and Other Materials: Some ROV video footage may become available. The D-ESP is also fitted with a CTD/transmissometer and ISUS. Data will be made publicly available.

Qualifications: Chris Scholin holds a PhD in Biological Oceanography from MIT/WHOI. He is a Senior Scientist and Chair of the Research Division at MBARI. Previous work in the sanctuary focused on application of the ESP in surface waters (harmful algal bloom research, etc).

Environmental Impacts: Deployment of this instrument will have minimal impact to the ocean floor. Approximately 2 m^2 of bottom will be disturbed during deployment and recovery of the instrument. All equipment will be recovered at the end of the experiment, and the ocean floor should rapidly return to its previous condition.

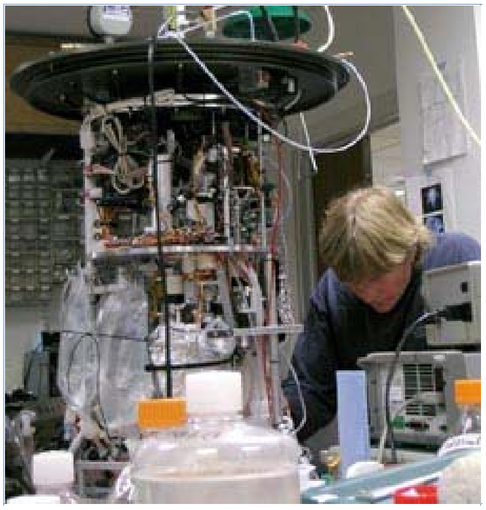
Copies of Other Permits: Earlier versions of the ESP have been routinely deployed in the Sanctuary under MBARI's general permit, MBNMS-2005-010 (see project 900612 in previous reports). A copy of this permit is included with this packet.

Figure 1. ESP undergoing trials in MBARI's test tank.

The MARS Ocean Observatory Testbed

Deep-Sea Environmental Sample Processor

An automated DNA lab looks for life in the deep sea



The Environmental Sample Processor (left) has to have all the chemicals, containers, and computers of a full-sized laboratory. Inventing ways to pack them all into a waist-high cylinder keeps Chris Scholin (right) and his colleagues busy. Image: Kim Fulton-Bennett, MBARI.

Two divers check out the second-generation ESP in MBARI's test tank before an early 2006 deployment. The ESP works well identifying proteins and other gene products in surface waters. The design team is now working to make the system function at 90 times surface pressure, and to allow the ESP

The environmental sample processor (ESP) is a molecular biology lab packed (robotic technician and all) inside a canister the size of a kitchen garbage can. A decade in development, the present version of the robot works well in surface waters. For the MARS observatory, MBARI is developing a deep-water ESP that can do the same work in the deep ocean's cold and extreme pressure.

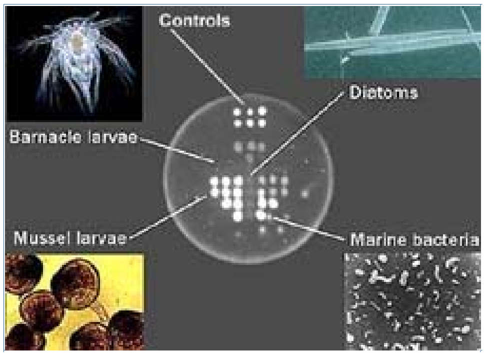
Underwater chemistry, done by a robot
At prearranged times or when triggered by another sensor, the ESP filters 2-L (a half-gallon) of seawater into its metal-alloy housing. The sample is washed and treated with chemicals that break down cell membranes. The freed cell contents are then washed over a wafer and passed to a series of robotic devices.

Within these devices is where the molecular chemistry happens. Standard tests detect gene products and answer specific questions: By detecting specific RNA sequences, the system can find out which organisms are present. Or, by testing for particular proteins, it can ask what those organisms are doing. (For example, an RNA test can look for a particular kind of harmful algae; a protein test can learn how much toxin it is producing.)

In surface waters, these tests are useful for monitoring algae and bacteria populations. They can also identify larger creatures such as mussel and barnacle larvae, helping biologists piece together normally hidden parts of these animals' life cycles.

The deep-sea ESP will be more of an explorer. Cold seeps on the floor of Monterey Bay harbor strange clams that use bizarre symbiotic bacteria to adsorb food. A deep ESP connected to the MARS observatory could detect DNA from these odd creatures, as well as look for other, completely new life forms. Later, the deep *to sequence DNA. Image: Todd Walsh, MBARI.*

ESP could go even deeper, to hydrothermal vents at twice the depth or more of MARS. Present methods for studying these vents center on using simple machines to collect and store samples until they can be hauled up and transported to a lab. A deep microbiology lab at a cabled observatory would go further, analyzing the sample as soon as it was collected and transmitting the results back home.



After the ESP has finished its work, the results literally shine through. Luminescent labels on an analysis wafer (center) are photographed. Molecules representing mussels, bacteria, other organisms, or lab controls bond to particular regions of the puck, so their location gives their identity away. Brightness indicates how much of a particular molecule was present. Image: Chris Scholin, MBARI.

The biggest obstacle to designing a deep ESP lies in doing standard labwork (normally done at around 21ºC (70ºF) and 1 atmosphere of pressure) on sea water that is about 4ºC (40ºF) and 90 atmospheres. MBARI engineers have had to construct an add-on module that collects sea water and depressurizes it before piping it into the main ESP compartment.

While engineers are redesigning the ESP's plumbing to make it deep-sea-worthy, the scientists are also expanding its analytical capabilities. Their goal is to be able to detect rare fragments of actual DNA. The analysis is routine for technicians in roomy, well-lit biology labs. But the steps involve heating, cooling, and reheating samples to precise temperatures – difficult tasks to automate in a small, closed space with limited power availability.

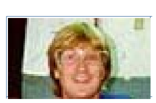
Why MARS?

As the 2004 discovery of *Osedax* whalebone-eating worms illustrated, Monterey Bay's depths still hold plenty of secrets. A MARS-based molecular biology lab will be able to sample deep bay water more frequently and with more flexibility in timing than a pre-programmed ESP running on battery power. Scientists can use other MARS sensors to decide when the deep ESP should take samples. And with the MARS site just 32 km (19 miles) by sea from MBARI, ship visits to resupply the deep ESP are easy to schedule.

Pushing the frontiers of automated microbiology down to the deep sea is fraught with difficulties, and key to the deep ESP's development is the MARS observatory's role as a testbed. The permanent connection to shore allows engineers to solve problems from their MBARI desks, rather than making repeat visits to sea to check on progress.

The MARS testbed is also key in developing the NEPTUNE cabled observatory. NEPTUNE envisions using multiple ESPs for long-term studies of the deep hydrothermal vents along the edges of the Juan de Fuca plate. Much of the hardware that will link NEPTUNE instruments and cables is under development at MARS. The deep ESP will also be part of a new microbial research center, called C-MORE (the Center for Microbial Oceanography: Research and Education) planned for the deep ocean off Hawaii. Designing and testing new instruments like the deep ESP is one of the MARS observatory's key services.

Related links



Dr. Chris Scholin
MBARI Senior Scientist

Leader of the ESP design team

- ESP project home page
- Evolution of a Robotic Underwater Biochemical Laboratory [600 kb PDF] – technical report about the second-generation ESP
- Results from ESP projects in the Gulf of Maine and Monterey Bay
- Robotic DNA lab helps scientists – MBARI homepage news story
- NASA's *Astrobiology* magazine covers the ESP's prospects at hydrothermal vents

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