Searching for Life in Extreme

A compact device will help identify new marine organisms and improve instruments for space exploration.

IN the scientific search for new life forms, nature continues to surprise, revealing marine species below freezing ice floes or next to scalding vents on the ocean floor. The Monterey Bay Aquarium Research Institute (MBARI), a nonprofit organization in Moss Landing, California, is one of several institutions developing instruments to aid the search for aquatic life forms and to further scientific research on microbes' role in mediating the cycling of Earth's elements and energy.

Marine microbes such as archaea, bacteria, and blue-green algae have significant effects on ocean chemistry and larger marine organisms. Studying these organisms is difficult, often because they cannot be cultured in the laboratory and have only recently been discovered. Possibly thousands of additional species have yet to be found.

Livermore scientists and engineers have joined the MBARI search for new life forms by designing an autonomous electro-opto-mechanical device that detects microbial genes of interest and measures their preponderance. The device, a polymerase chain reaction (PCR) module,

Livermore engineer Vincent Riot holds the polymerase chain reaction (PCR) module developed for research in extreme environments from the seafloor to other planets. (background) An artist's concept shows a space probe on Europa, one of Jupiter's natural satellites. Such a probe, equipped with a PCR module, would penetrate Europa's icy surface and descend through its liquid ocean to search for life near volcanoes or hydrothermal vents. (Rendering courtesy of the National Aeronautics and Space Administration.)

Environments

incorporates features from Livermore-designed systems that detect pathogens, but it is much more compact and "intelligent" than previous instruments.

Data produced by the PCR module will help researchers better understand the roles microbes may play in responding to global climate change. Scientists estimate that the world's oceans are absorbing about one-third of the carbon dioxide produced by burning fossil fuels, gradually causing seawater to become more acidic. As part of Earth's carbon cycle, species of microscopic marine algae take up large quantities of carbon dioxide and release oxygen. By studying the genetic makeup of these species, scientists can learn how microbes remove carbon from the atmosphere and cope with the increasing acidity of oceans.

The combination of PCR data with results from other instruments developed to investigate marine environments will also be evaluated as part of a National Aeronautics and Space Administration (NASA) program to determine the instrumentation to deploy in searching for life on other planets. Livermore researchers are developing the PCR module as part of NASA's Astrobiology Science and Technology for Exploring Planets Program.

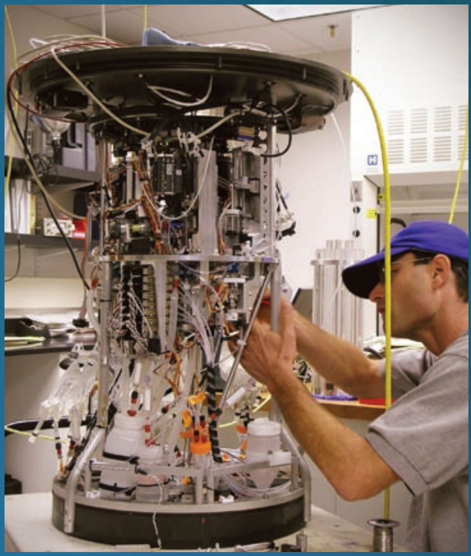
By any measure, one of Earth's extreme environments is the deep sea, where superheated (more than 350°C) fluids mix with near-freezing seawater at several hundred atmospheres of pressure, where 1 atmosphere equals more than 100 kilopascals. Compared to most of the deep sea, the areas immediately around hydrothermal vents typically support complex microbial communities. Deep-sea studies may also contribute to astrophysics research because extreme environments on Earth could be similar to conditions elsewhere in the solar system. For example, the surface of Europa (a Jupiter satellite) appears to be entirely submerged beneath a sea covered with thick ice. Some scientists speculate that primitive life forms resembling Earth's microbes could exist around active volcanoes or hydrothermal vents believed to exist on Europa's seafloor.

Remote Sampling in the Ocean

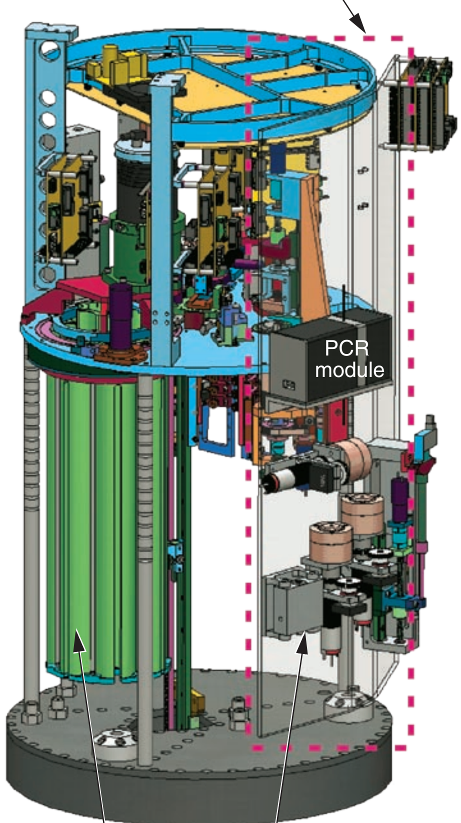
To analyze marine organisms in their native habitats, MBARI scientists developed the Environmental Sample Processor (ESP). This remote instrument system collects water samples and puts them through several stages of filtration to obtain a variety of microbes. The processor lyses, or dissolves, the filtered cells to obtain DNA, RNA, and proteins. It then forwards concentrated extracts to instruments that analyze the genetic material and identify the microbes and their gene products, such as deadly marine toxins. ESP also archives samples for further analysis after the device is recovered and returned to land.

ESP consists of three major components: the core sample processor (or core ESP), a sampling module, and add-on analytical modules. The core ESP extracts target molecules from filtered particulate matter and performs DNA and protein array analyses. This component handles sample volumes from a few milliliters to several liters at depths to 50 meters. Below 50 meters, an external sampling module introduces depressurized seawater into the core ESP, which operates internally at about 1 atmosphere regardless of depth.

Analytical modules such as Livermore's PCR module are stand-alone systems that provide enhanced analysis downstream of routine sample-processing operations.



Electrical engineer Scott Jensen of the Monterey Bay Aquarium Research Institute (MBARI) works on the second-generation Environmental Sample Processor (ESP). A self-contained robotic laboratory, ESP collects water samples, concentrates microbes, and automates instruments that identify microbes and their gene products. (Courtesy of Kim Fulton-Bennett, MBARI, © 2005.)



This model shows the core ESP with a PCR module and microfluidic block attached on the right-hand side. (Courtesy of MBARI, © 2008.)

In addition to the three primary components, outside environmental sensors monitor temperature, depth, and salinity and measure the concentrations of chlorophyll, nitrates, dissolved gases, and other substances.

According to MBARI molecular biologist Chris Scholin, the limited number of sampling opportunities has hindered marine life studies. Organic material is often collected in brief surveys conducted by costly research vessels. The resulting data "snapshots" may not reflect the dynamics of fluctuating populations and metabolic changes that microbes undergo in response to changing environmental conditions.

To overcome the problems posed by typical at-sea scientific investigations, MBARI researchers have designed ESP to be a plug-and-play sample-processing device. "ESP is a lab in a can," says Scholin. "It can operate continuously for weeks to a month in the same location." With ESP, researchers can conduct biological analyses remotely, in real time, using the processor's interactive functionality. Its long-term deployment capability allows scientists to determine if certain types of microbes are present at a particular place and time and to archive samples for future analyses.

MBARI currently has six ESPs (including a deep-water version), each measuring about 0.5 meters wide by 1 meter tall. Units weigh 32 to 45 kilograms, depending on the instruments selected for the mission. In the past several years, Scholin and his team have used ESPs in experiments near the sea surface (within the upper 15 meters) in Monterey Bay to study microscopic marine life, such as bacteria, archaea, phytoplankton, toxins produced by algae, and small invertebrates.

In one project, they examined archaea, primitive single-celled organisms that contain no cell nucleus and live in hot springs and deep-sea vents. Archaea are important components of Earth's carbon cycle (in which carbon compounds move between air, land, and sea) and its nitrogen cycle (the process that transforms nitrogen and nitrogen-containing compounds in nature). Also of considerable interest is phytoplankton, or planktonic plant life, which encompasses a variety of microalgae that live near the water surface and absorb light for photosynthesis. Phytoplankton are important because they provide a major source of food to aquatic life.

Improving Data Fidelity

The assays deployed onboard every ESP include DNA and protein arrays, which identify selected organisms and metabolites. Although these tests are useful, identifying particular genes with high confidence requires the higher fidelity analyses provided by PCR, a technique widely used in molecular biology. PCR generates millions of copies of a particular DNA sequence for easy identification. The method relies on repeated heating and cooling cycles to quickly replicate the targeted DNA in the large quantities needed for research. However, because PCR does not test for all unknowns, scientists must decide what organisms to look for before processing begins.

The technique features short DNA fragments called primers, which contain sequences of nucleotides (the building blocks of DNA) that complement the DNA region targeted for study. As a sample is heated, its two intertwined strands of DNA unwind. During the cooling process, DNA makes a copy of itself through an enzyme called DNA polymerase, provided the primers find their complementary sequences in the sample. In one heating and cooling cycle, the amount of targeted DNA approximately doubles—a replication process that takes less than 2 minutes. Within 30 cycles, a single molecule of DNA is amplified more than 1 billion times.

A synthesized DNA probe tagged with a fluorescent dye is introduced between the primers. An increase in fluorescence indicates that the targeted DNA sequence has been detected. When more copies of the target are present, the signal is stronger. Each PCR channel is assigned a different color of light to process more than one type of DNA. Because PCR amplifies the regions of DNA that it targets, it can discriminate between slightly different species. For example, it can distinguish pathogenic from nonpathogenic strains of the same species.

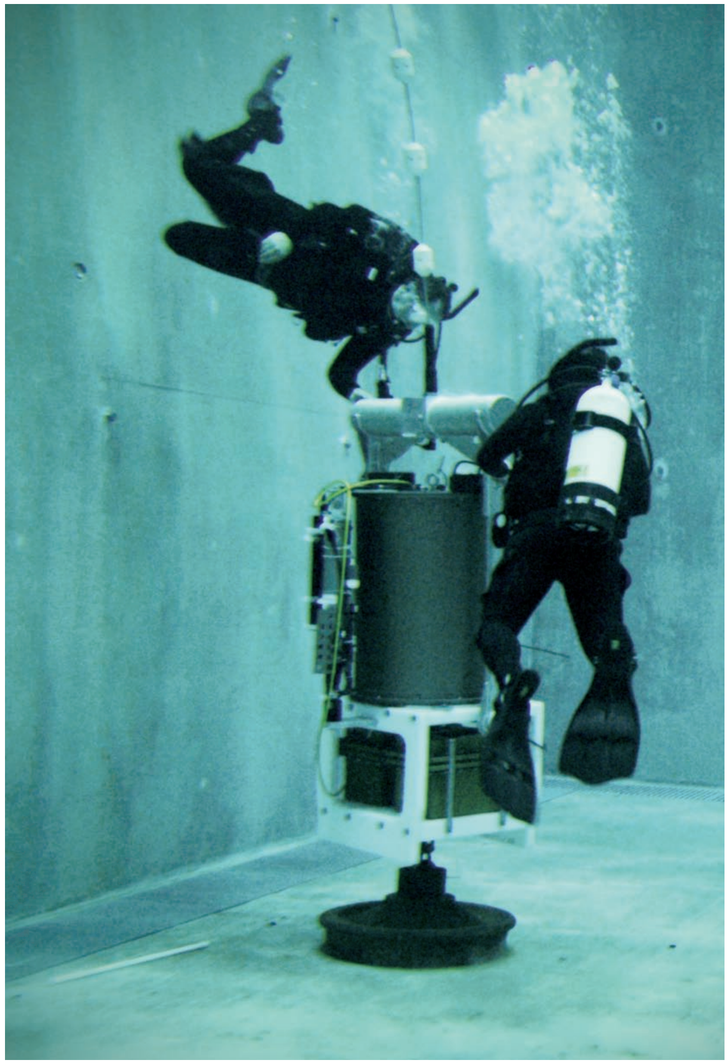
PCR is both more sensitive and more specific than the standard probe arrays. "With the DNA probe arrays on ESP, we can look for more targets simultaneously but with less sensitivity than PCR offers," says Scholin. "PCR allows us to search for target molecules that we can't find with other ocean-deployable instruments. We can study genes that regulate the nitrogen cycle, for example, or that are involved in a certain biochemical pathway. We can target a specific species or a general class of species that share the same gene. By combining DNA probe arrays and PCR, we can obtain a community profile and quantify the presence of particular genes."

Tapping PCR Experience

MBARI turned to Livermore researchers to design the compact PCR module because of their expertise with this technology. Over the past decade, the Laboratory has developed a number of PCR-based systems that rapidly detect and identify airborne biological agents. Among such systems are the handheld advanced nucleic acid analyzer and the autonomous pathogen detection system (APDS). APDS monitors the air for biological threat agents including bacteria, viruses, and toxins, and units can operate continuously in public areas such as subway stations.

"Our challenge was to develop a compact, low-power, smart system to run PCR reactions and take measurements," says Livermore chemical and mechanical engineer John Dzenitis, who led the PCR module development effort. "Running PCR underwater had never been done before. We were confident we could do it, though, because operating the sample processor is analogous to running an APDS unit underwater. Before we developed APDS, no one had autonomously operated this type of detector in a subway."

The performance goals for the PCR module were similar to those for an APDS unit, but the underwater device had to be self-contained. In addition, the Livermore team wanted to provide fast heating and cooling cycles and at least two optical channels for reading concentrations of different fluorescent-labeled molecules. The team also had to ensure that the device drew minimal power from ESP's marine batteries and interfaced seamlessly with the processor's fluid-shuttling systems. Finally, the module had to be easy to program and able to function without receiving external commands.



Divers test an ESP in a deep-water tank prior to ocean deployment. (Courtesy of Todd Walsh, MBARI, © 2006.)

To achieve these goals, team members focused on specific aspects of the module design. Bill Benett worked on mechanical design and low-power heater fabrication, while Dean Hadley developed the analog electronics. Former Laboratory engineer Tony Makarewicz was responsible for optics and fluidics integration, and Vincent Riot designed the digital electronics and the related software. "Vincent's electronics and software really set this PCR module apart from previous designs," says Dzenitis. "The controller offers a simple but flexible command set and has proven to be very reliable."

The Livermore team assembles each device, programs the software, and checks out the system before transferring a module to MBARI. A master controller and subsidiary processors allow the module to operate without external commands. Custom software instructs each channel what to do, including the number of heating and cooling cycles to run for different samples.

Module components are enclosed in a black box measuring 13 centimeters long by 7 centimeters high by 5 centimeters wide and weighing about 425 grams. (See the figure below.) One or two units can fit easily inside an ESP. Current designs feature two PCR channels with the capacity for two additional channels, one of which can be used as a control. Module interfaces include 12-volt direct-current power, tubing for samples and reagents, and data transmission.

In operation, the PCR module receives a concentrated extract of DNA taken from marine microbes. The module then amplifies the target DNA molecules, measures their fluorescence, and reports the results. The device requires one to two hours from the time it receives a sample to confirm the presence of a specific gene or section of DNA.

In fall 2008, MBARI deployed a prototype PCR module in an ESP suspended to a depth of 10 meters about 10 kilometers offshore in Monterey Bay. The module was fielded a second time in May 2009, also off the Monterey coast. Both missions lasted about a month. An electromechanical cable connected to a radio modem on a surface float transmitted commands and data. In both field tests, the PCR module successfully identified a series of target genes.

Active and Sleep Modes

Unlike Livermore's APDS, the underwater ESP does not operate the PCR module continuously. Instead, the module runs intermittently based on a programmed schedule. After sample processing is complete, the module reverts to "sleep" mode to conserve power. MBARI scientists and engineers are developing the capability to autonomously trigger specific PCR analyses in response to an external event, such as a sudden change in seismic activity, temperature, or water chemistry. Says Riot, "If needed, the module could process samples every day at the same time, or if conditions dictate, do many reactions for a few days, or follow any other schedule."

Water samples are processed within the core ESP by metal disks called pucks, measuring 30 millimeters in diameter and 17 millimeters tall. The pucks serve as surrogates for a traditional laboratory bench. Located in a rotating carousel, the pucks collect and homogenize large-volume samples and process probe arrays for nucleic acid and protein analyses. Pucks also can preserve samples for later analysis on shore.

Once microbes are filtered, chemicals break down their cell walls and release their genetic material into solution for testing. Samples then pass over a puck imprinted with an array of tiny dots that contain chemical mixtures and fragments of DNA probes used to identify organisms. In a similar process, protein arrays detect certain proteins such as domoic acid, an algal neurotoxin that can be deadly to marine life and humans.

The PCR module requires more extensive sample preparation than that needed by the DNA and protein arrays. To meet that need, the Livermore team helped MBARI design a separate fluid-handling system for the core ESP. Called a microfluidic block, this device distributes samples and reagents via a series of valves and pumps. It also takes

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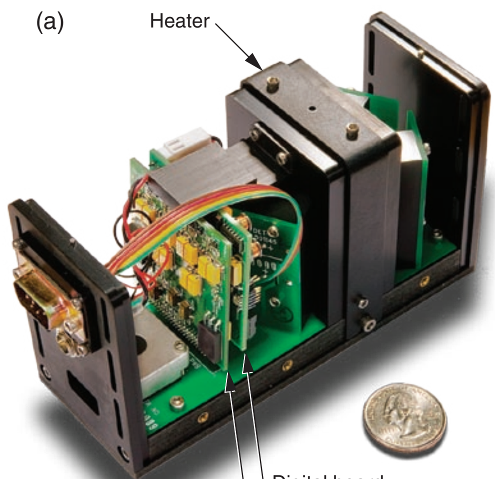

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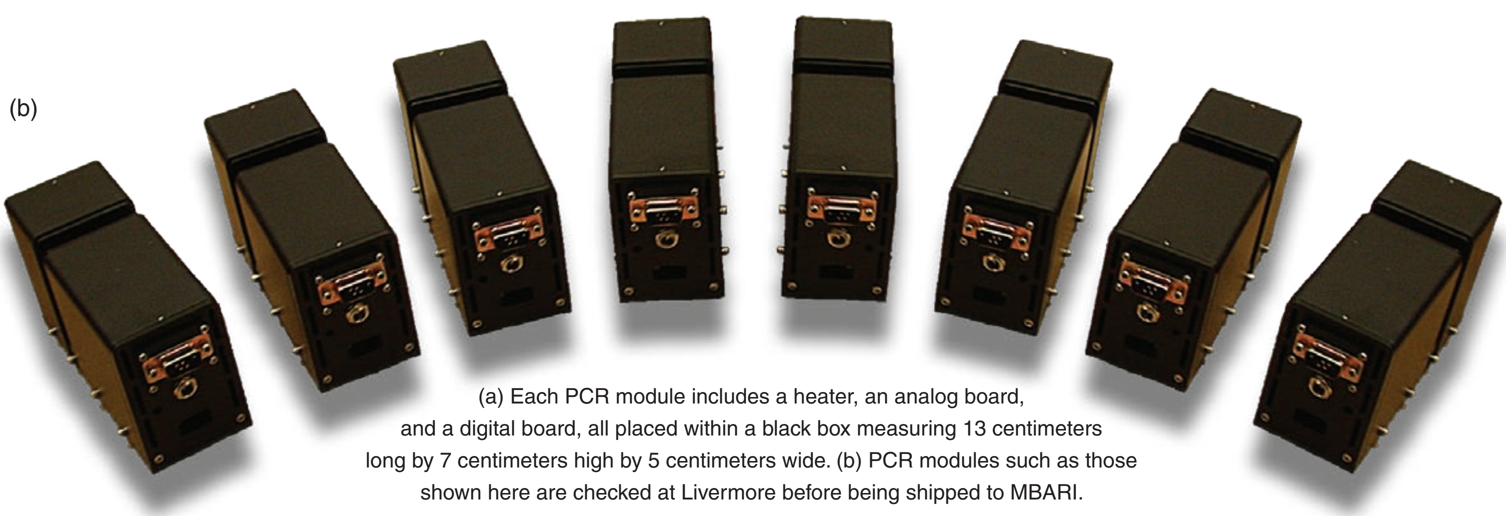


(a) Each PCR module includes a heater, an analog board, and a digital board, all placed within a black box measuring 13 centimeters long by 7 centimeters high by 5 centimeters wide. (b) PCR modules such as those shown here are checked at Livermore before being shipped to MBARI.

crude sample homogenates from the core ESP and processes them, for example, purifying the DNA. It then combines purified DNA with chemicals and shuttles microliter quantities of the mixture into the PCR module. “Like the fluidics module in APDS, the microfluidics block is an automated platform,” says Dzenitis. “It follows the same steps a biologist performs with pipettes in preparing samples for a benchtop PCR instrument.”

Designed for Deep Water

Although the PCR module can be used on all ESPs, it was designed with Deep ESP in mind. This version of the processor, which can operate in waters as deep as 4,000 meters, includes an original ESP surrounded by a 1-centimeter-thick titanium pressure housing. The Deep ESP sampling module can take in up to 10 liters of water at pressures of up to 400 atmospheres. It then decompresses the water to about 1 atmosphere and pumps it into the core ESP. Together with its sampling module and support frame, Deep ESP weighs several thousand kilograms. Underwater, however, it is only about 45 kilograms because of buoyancy provided by air in the pressure housing and in blocks of special flotation foam bolted to the top of the processor’s frame.

Deep ESP can be placed on the sea bottom and hooked to a cable carrying power and data from shore or a buoy and linked by satellite to researchers on shore. MBARI researchers ran the first samples through a Deep ESP in May.

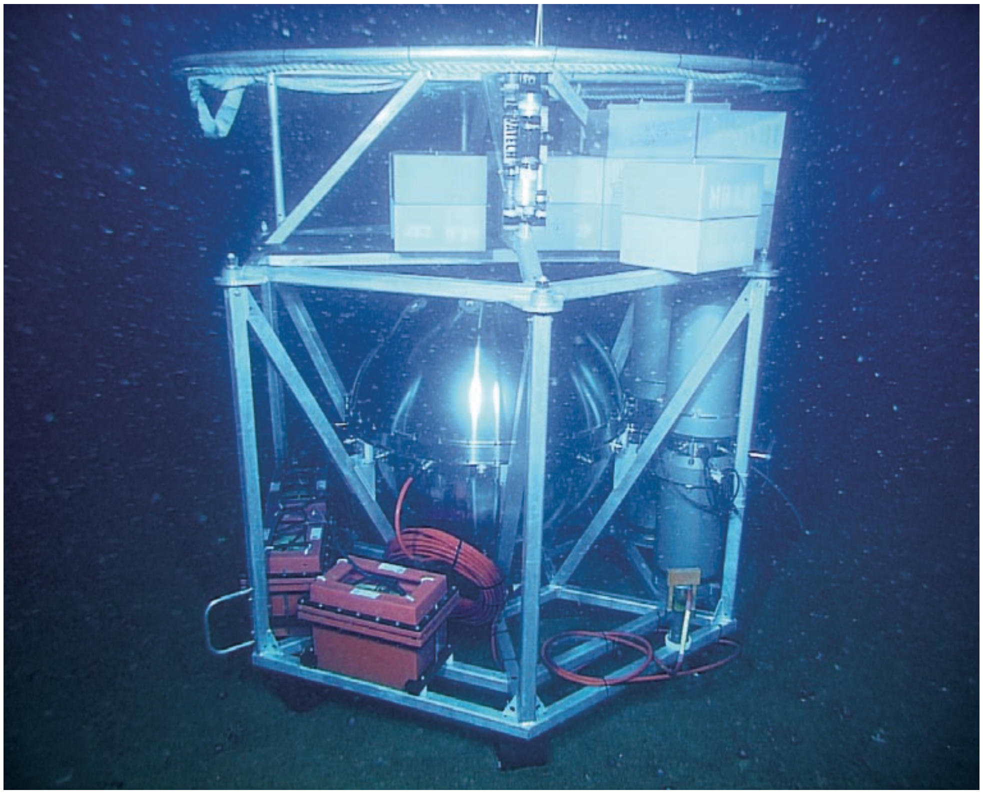
Scientists are especially interested in using Deep ESP to study organisms that have evolved over millions of years to survive in such extreme environments as the methane seeps in the Santa Barbara Basin or the hot, deep-sea vents found at the Axial Seamount submarine volcano off the Oregon coast. The MBARI team has planned a Deep ESP expedition near the Axial Seamount for 2009 to study the interaction between volcanic events and thriving microbial populations. Another potential study environment is adjacent to methane hydrate (“fire ice”) outcroppings off California, Oregon, and Washington. These outcroppings, which consist of methane trapped within ice, support large microbial populations.

In its first sea trial, Deep ESP is lowered into Monterey Bay from the research vessel Point Lobos. Designed for depths to 4,000 meters, the instrument is enclosed in a titanium pressure vessel and protected by a strong support frame. Several blocks of flotation foam are secured to the top of the frame. (Courtesy of MBARI, © 2009.)

The Livermore team delivered three PCR modules to MBARI in early 2009, and another eight units are in assembly. Later this year, a Deep ESP containing the PCR module will be connected to the Monterey Accelerated Research System. This undersea observatory includes a 52-kilometer cable that carries data and power to an electronics package called a science node located 890 meters below the surface of Monterey Bay. With this system, researchers can remain onshore while they run experiments and gather data.

Growing Interest

MBARI scientists report increasing interest worldwide in using ESPs. Researchers have proposed applying the processors to study ecologic relationships among the thousands of marine microbe species, to detect harmful or toxic microbes for monitoring water quality and managing water resources, and to explore other areas of extreme environments. Data gained from ESP research will likely help scientists improve their understanding of Earth’s oceanic processes, many



In initial tests, Deep ESP operated on the seafloor about 640 meters below the surface. (Courtesy of MBARI, © 2009.)

of which are strongly influenced by microbial communities.

The Laboratory, meanwhile, has received interest in licensing its compact PCR technology. Livermore's Industrial Partnerships Office is currently in license negotiations with Spyglass Biosecurity, Inc., a small San Francisco startup company.

The advances incorporated in the PCR module may soon be added to other Livermore devices. For example, in current APDS units, interactions between the master controller and the components it regulates can be troublesome. On the next version of APDS, Livermore designers plan to offload the control for most functions to smart electronic cards. Such a change, says Riot, would simplify APDS operation and make the instruments more robust.

Scholin notes that the development of ESP and its deep-water version, along with PCR and other analytical modules, provides NASA with a model for developing a compact, low-power device to search the solar system for signs of life. "NASA faces many of the same problems ocean scientists do in terms of sample acquisition, preparation, autonomous operation, and data transmission," he says.

Dzenitis adds that the Livermore researchers are proud that they could tap national security expertise to contribute to efforts for monitoring the health of the oceans, discovering life on Earth—and perhaps exploring other worlds, as well.

—Arnie Heller

Key Words: archaea, Astrobiology Science and Technology for Exploring Planets Program, autonomous pathogen detection system (APDS), Environmental Sample Processor (ESP), Europa, Monterey Bay Aquarium Research Institute (MBARI), polymerase chain reaction (PCR) module.

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