The Environmental Sample Processor (ESP): A Device for Detecting Microoganisms *In Situ* Using Molecular Probe Technology

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http://www.mbari.org/microbial/ESP/

LONG-TERM GOALS

Molecular diagnostic procedures for identifying water borne microorganisms, genes they may harbor and express, and toxins they may produce play a central role in many research and resource management activities throughout the U.S. and elsewhere, but such methods generally require the return of discrete samples to a laboratory for analysis. The long-term goal of this project is to develop an *in situ* instrument system that allows us to overcome this impediment. Towards that end we are exploring use of and further developing the Environmental Sample Processor (ESP), a novel instrument that collects discrete, subsurface water samples remotely and utilizes molecular probe technology to detect a wide range of microorganisms and substances they produce. We aim to deploy an array of internet-accessible ESP's and carry out a variety of "wet-chemistry" molecular biological analyses in support of basic environmental research and resource management activities, such as those consistent with a variety of ocean and water shed observing initiatives (e.g., OOI/ORION, IOOS, GOOS, NEON, GEOHAB, OHH, etc.)

OBJECTIVES

The "first generation" (1G) ESP was built by the Monterey Bay Aquarium Research Institute (MBARI) and was used to prove basic concepts related to collecting water samples autonomously, concentrating microorganisms in those samples, applying DNA probes to identify and quantify of particular species captured, and data telemetry (Scholin et al., 1998, 2001, in revsion). In addition, the 1G ESP was designed to archive discrete samples for nucleic acid, microscopic and toxin analyses for

validating real-time data from the probe arrays as well as facilitating other analyses in the laboratory (e.g., construction of gene libraries). More information can be found on the web at http://www.mbari.org/microbial/ESP/. The 1G ESP worked successfully on several, limited test deployments with extensive support by the original science/engineering design team. Development of a "second generation" (2G) ESP began with this project, with support from the National Oceanographic Partnership Program (NOPP) through funds allocated by the National Science Foundation (NSF, OCE-0314222). In addition, the 2G ESP is supported by MBARI through funds allocated by the David and Lucille Packard Foundation. The overall goals in designing the 2G ESP are to make this instrument much more robust and user friendly than the original prototype, to reduce its size, complexity and power consumption, and to take advantage of microfluidic-scale molecular detection technologies. Finally, the project team aims to make ESP available to the oceanographic research community at-large.

APPROACH AND WORK PLAN

1) Technical approach

Functional requirements of the 2G ESP were derived from our experience in developing and deploying the 1G ESP, and also based on input from a group of researchers and resource managers that attended the ESP workshop held at MBARI in January 2003. Workshop participants included people from MBARI as well as other institutions (academic, non-profit, government), scientists and engineers, and those who could be among the first end-users of the 2G ESP as an operational sensor system. Applications of the ESP discussed at the workshop varied from shallow, fresh and brackish waters, to coastal regions, to the deep-sea. Organisms identified as indicator species for which the ESP would be used to either detect *in situ* or archive included relatively large invertebrate larvae, phytoplankton, and cyano-, archae- and eubacteria. Despite the broad range of proposed deployment environments and the spectrum of sample types envisioned, workshop participants agreed that many requirements for sample processing (e.g., sample archival and homogenization, solid phase extraction chemistries, etc.) were very similar and could be met by a single, core instrument: the ESP. Special requirements associated with collecting large volumes or sampling at great depth, etc., must be met by hardware external to the ESP. Such devices were termed "Sampling Modules". Detection of analytes not possible in the current, core ESP design could be met by the future addition of stand alone devices internal to the ESP that take advantage of the ESP's sample acquisition and processing capabilities. The latter were termed "Analytical Modules". The preliminary design of the core 2G ESP was thus based on those concepts: core sample processing, sampling modules, and analytical modules. The focus of the NOPP/NSF project is to build and deploy the core 2G ESP.

The heart of the ESP design revolves around the use of "pucks" (Figure 1), reaction chambers that allow a common electromechanical, fluid handling device to carry out a diverse set of functions, from filtering water and collecting particles to developing DNA and antibody probe arrays, etc. Chemical reagents can be trapped in a puck so that specific reactions can occur over an extended period at a defined temperature. Target molecules eluted from one puck may be retained and used in a subsequent protocol, thereby enhancing the instrument's analytical efficiency. Development of pucks to allow precisely controlled chemical reactions to occur in an ocean going sensor has been a major part of our design effort.

Figure 1. ESP "pucks" that hold 25 mm user-defined media. Pucks are highly configurable for performing different types of analyses, but all conform to a common outer envelope (bottom row). The three pucks shown (one puck per column shown assembled in bottom row with top and bottom halves above) are used for (left to right) creating cell homogenates, archiving cells for microscopy, and developing DNA probe arrays. All utilize a common core processor, the ESP, for carrying out those functions. Pucks are stored in rotating carousels and loaded/unloaded into the sample collection and processing system using a mechanical actuator.

2) Key individuals participating in this work.

The project team consists of scientists and engineers from two different laboratories: MBARI (Moss Landing, CA) and the Marine Biotoxins Program, NOAA/NOS (Charleston, SC) as detailed below:

MBARI

Christopher Scholin – P.I. – Science behind sample collection, processing, and application of molecular probes for detection of microorganisms.
Eugene Massion – Co-P.I. – Mechanical engineer.
Jason Feldman – Mechanical engineer.
Scott Jensen. – Electrical engineer.
Brent Roman. – Software engineer.
Joe Jones – Science technical support: application of the ESP; invertebrate larvae.
Roman Marin III – Science technical support: application of the ESP; phytoplankton.
Christina Preston – Science technical support: application of the ESP; microbes.

NOAA/National Ocean Service

Gregory Doucette – Co-P.I. – Science behind sample collection, processing, and application of molecular probes for detection of algal toxins.
Christina Mikulski – Science technical support: application of the ESP; algal toxins.
Kristen King – Science technical support: application of the ESP; algal toxins.

3) Work plans for 2005.

Specific plans for 2005 are as follows:

1) Complete integration and laboratory engineering/science testing of the 2G ESP (includes validation of all science protocols and sensor calibration for field use).
2) Complete pressure housing and mooring for deploying the 2G ESP.
3) Deploy the 2G ESP in surface waters of Monterey Bay (by May, 2005).
4) Conduct time series analyses using the ESP with real-time, molecular probe-based detection of invertebrate larvae, harmful algae and marine microbes, and domoic acid (algal toxin).
5) Complete engineering documentation of the instrument.

6) Submit manuscripts and attend conferences/give presentations related to the ESP.

WORK COMPLETED

Design, fabrication and testing of many of the core mechanical and electrical components have been completed. A solid model overview of the 2G ESP is shown in Figure 2. Integration of the mechanical system with the electronics and software is on-going, as is completion of the low power command/control interface. The science team has been developing and refining their chemistry protocols using a test fixture that allows them to process samples manually in a format consistent with that of the actual ESP so that progress can be made without access to the real instrument. Protocols for sample collection, archival, homogenization were successfully transitioned to the actual 2G ESP hardware, proving that the system meets or exceeds its specifications. In some instances, small changes to the design were necessary to optimize performance (e.g., changing specific valves and fittings or placement of components was necessary once the prototype was built and tested). The science and engineering teams are now in the process of integrating the microfluidic sample processing stage along side that of the sample collection stage. Testing of the complete fluidic system will begin in February.

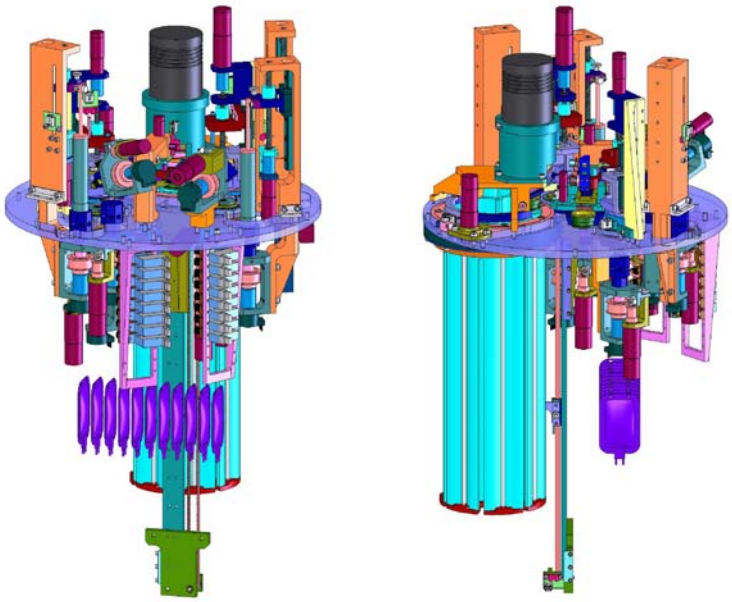

Figure 2. Solid model of the 2G ESP showing two side views of the instrument (structural elements omitted for clarity). Actual size of the instrument is ~16 x 30 inches (diameter x length). The 2G ESP is built around a rotating carousel (e.g., right image, light blue cylinder) that holds "pucks" (e.g., Fig 1). An elevator and mechanical actuator are used to load/unload pucks into the sample collection and processing stages of the ESP. Syringe pumps (orange structures) plumbed to a variety of rotary and solenoid valves allow for sample collection and chemical reagent application. Solutions used to process samples are stored in bags (e.g., left image, visible as a series of purple objects). A CCD camera (dark cylindrical object visible at top of the instrument) records results of DNA and antibody probe array tests.

RESULTS

The project is slightly behind schedule but well within bounds of the proposed objectives. It took a more time than anticipated to hire a qualified mechanical designer to translate the instrument's conceptual design and functional requirements into actual machine prints and assembled hardware. The ESP's command and control system is based on a combination of off-the-shelf and custom electronics, resulting in a very low power (~2W), modular solution for actuating mechanical parts, effecting closed-loop control (e.g., motor position and temperature controls), etc. The software interface is based on a very flexible, easy to use, high level scripting language known as Ruby running under the Linux operating system. Integration of the mechanical, electrical, and software aspects of the project has proceeded to the point where the science team is now writing Ruby scripts to control core pieces of the instrument (e.g., to automate sample collection and processing by actuating a series of valves, syringes, and by controlling temperature of the puck). In addition, we uncovered a series of material incompatibility problems that for several reasons impaired the performance of the 1G ESP. Gaining an understanding of these 'gottchas' lead us to rigorously test all materials specified for the 2G ESP that would come in contact with samples and reagents, again adding extra work that was not expected. Despite these set-backs, the team has made remarkable progress, and in approximately 1.5 years moved from a relatively crude concept drawing to a complete SolidWorks model (e.g., Figure 2), developed a nearly complete set of machine prints, have most parts in-hand, and are well underway with engineering integration and testing of the first major pieces of the instrument. We expect to build up at least 1-2 additional copies of the instrument by the end of 2005.

Rather than rush to a stand-alone field deployment prior to December '04 as we had originally hoped, we decided it much more prudent to emphasize testing the completed 2G ESP in the lab, test tank, and raw water tank, and then conduct extensive field trials in Monterey Bay, CA, during spring and summer 2005. For that reason, we pressed the existing 1G ESP into service August-October 2004 as a way to field test some of the newer molecular analytical protocols destined for deployment on the 2G ESP. We have succeeded in fielding three different classes of DNA probe arrays for use in a single deployment, targeting groups of marine microbes, several species of harmful algae, and invertebrate larvae (mussels, barnacles). Reagents used in these analyses are stable for at least 6 months at room temperature. We are currently developing an antibody-based test for domoic acid (an algal toxin responsible for illness of humans and wildlife) that will also run on the ESP, *in situ*.

In support of technology transfer, we are pursuing extramural funding to build up a limited ESP instrument pool, and are working with a few qualified external research groups to integrate the 2G ESP into their research activities. Areas where the ESP will likely find immediate application outside of MBARI/Monterey Bay include Gulf of Maine, Gulf of Mexico, the Northeast Pacific (NEPTUNE study area), and the HOTS site (University of Hawaii).

IMPACT AND APPLICATIONS

National Security

Some design concepts and/or components of the ESP could be applied towards detection of water borne, bioterrorism agents.

Economic Development

The ESP, as well as systems that may evolve from it, could be commercialized and sold to researchers, resource managers, and various government agencies. Commercialization of the ESP system would likely also involve production of "reagent packs" (e.g., similar to ink cartridges for a computer printer), service and operations contracting, and consultation for customizing the ESP sensor system.

Quality of Life

Rapid and specific detection of water borne microorganisms is a cornerstone of numerous research and resource management activities. ESPs could make it possible to detect a wide array of organisms that pose risks to humans, wildlife, and ecosystems. These sensors could operate *in situ* in concert with other observing systems/platforms. This strategy would provide a unique capability for collecting discrete samples synoptically, carrying out sophisticated molecular analytical analyses autonomously, and transmitting data obtained to a central location for processing, interpretation, and dissemination.

Science Education and Communication

The ESP project offers tremendous opportunities for teaching and outreach. For example, the ESP project spans such varied topics as engineering/instrumentation development, identification and description of life history stages for particular species, ecology/food webs, toxicology, bloom dynamics, physical/chemical oceanography, resource management, land use practices, policy making, and economics.

TRANSITIONS

Quality of Life

Many investigators outside of MBARI have approached us as to how they might access and use the ESP to facilitate their research, all having immediate applications of the instrument broadly falling under the heading of emerging coastal ocean observatory programs targeting harmful algal blooms and microbial populations in particular. We are actively soliciting funding to support such emerging partnerships with the long-term goal of transitioning the ESP technology to these and other end-user groups.

Science Education and Communication

We are disseminating information about the ESP by participating in relevant conferences and workshops, submitting manuscripts for peer-reviewed publication, and by posting information on the web. Education opportunities related to the ESP exist for postdoctoral, graduate and undergraduate students.

RELATED PROJECTS

The DNA probe array project at MBARI (see http://www.mbari.org/microbial/hab/), and the "deep-sea ESP (D-ESP; see http://www.mbari.org/microbial/ESP/deep_ESP_frontpage.htm).

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PUBLICATIONS

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