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

P.I. Dr. Christopher A. Scholin
Monterey Bay Aquarium research Institute
7700 Sandholdt Rd.
Moss Landing, CA 95039-0628
Phone: (831) 775-1779 FAX: (831) 775-1620 E-mail: scholin@mbari.org

Co-P.I. Eugene Massion
Monterey Bay Aquarium research Institute
7700 Sandholdt Rd.
Moss Landing, CA 95039-0628
Phone: (831) 775-1779 FAX: (831) 775-1620 E-mail: magene@mbari.org

Co-P.I. Dr. Gregory J. Doucette
Marine Biotoxins Program, NOAA/National Ocean Service
219 Fort Johnson Rd.
Charleston, SC 29412
Phone: (843) 762-8528 FAX: (843) 762-8700 E-mail: greg.doucette@noaa.gov

Award Number: NSF OCE-314222

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

LONG-TERM GOALS

Molecular diagnostic procedures for identifying water-borne microorganisms and for elucidating the roles they play in biogeochemical cycles are central to many research and resource management activities throughout the U.S. and elsewhere. However, such methods generally require the return of discrete samples to a laboratory for analysis at a later time. The primary goal of the Environmental Sample Processor (ESP) project is to develop an *in situ* instrument that allows us to overcome that impediment by enabling autonomous sample collection and application of molecular probe technology to detect water-borne microorganisms remotely (http://www.mbari.org/microbial/ESP/). A longer-term goal is to deploy an array of internet-accessible ESP's in support of basic environmental research and resource management activities consistent with a national and international ocean and watershed observing initiatives such as OOI/ORION, IOOS, GOOS, NEON, GEOHAB, OHH, etc.

OBJECTIVES

A "first generation" ESP (Scholin et al 2001) was deployed in Monterey Bay and the Gulf of Maine, demonstrating DNA probe array-based detection of a variety of organisms (e.g., Goffredi et al 2005, Scholin et al. in press). Based on that work, development of a "second generation" (2G) ESP is being carried out with support from NOPP through funds allocated by the NSF (OCE-0314222) and the Monterey Bay Aquarium Research Institute (MBARI). The objectives of this project are to:

1) Develop a detailed design for a "second generation" (2G) ESP.

2) Construct the 2G ESP and a mooring system for its deployment in the ocean.
3) Refine DNA probe array technology for detecting individual species using the ESP.
4) Develop a capability for detecting algal toxins using the ESP.
5) Conduct field tests of the 2G ESP in Monterey Bay, California.
6) Initiate transfer of the ESP technology and operational know-how to researchers and resource managers outside of the immediate project team.

Detection of specific nuisance, harmful or toxic algal species and their bioactive compounds (e.g., toxins) that pose widespread economic concerns and/or are known to negatively impact the health of humans and wildlife is emphasized.

APPROACH AND WORK PLAN

<u>Technical Approach</u>
The technical specifications and detailed design of the 2G ESP were based on experience gained from fielding the 1G device, as well as input from a group of researchers and resource managers that attended a workshop held at MBARI in January 2003. Compared to the 1G prototype, the design objectives underlying the 2G ESP are to make this instrument much more robust and user-friendly, to reduce its size, complexity and power consumption, and to take advantage of microfluidic-scale molecular detection technologies.

<u>Key individuals participating in this work - 2005</u>
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.
Dianne Greenfield – Postdoctoral Fellow: application of the ESP; phytoplankton.
Christina Preston – Science technical support: application of the ESP; microbes.
Roman Marin III – Science technical support

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.

<u>Work plans for 2006</u>
Specific plans for 2006 are as follows:

1) Deploy the 2G ESP in surface waters of Monterey Bay (Q1-3, 2006).

2) Conduct time series analyses using the ESP with real-time, molecular probe-based detection of marine invertebrate larvae, harmful algae and microbes, as well as the algal toxin domoic acid.
3) Construct copies of the 2G ESP to facilitate technology transfer (Q3-4, 2006)
4) Hold a “ESP users workshop” for a selected group of researchers that have expressed an interest in using the instrument in support of their own research programs (Q4, 2006)
5) Submit manuscripts and attend conferences/give presentations related to the ESP work completed.

WORK COMPLETED

Design, fabrication and testing of the core 2G ESP mechanical and electrical components, pressure housing, and a mooring for deploying the device in the ocean, have been completed (Fig. 1). Protocols for sample collection, archival, homogenization and DNA probe array development have been transitioned to the new, fully integrated device. Protocols for detecting domoic acid using an antibody-based assay have been worked out at the lab bench level using isolated ESP components, and these procedures are now being translated to the 2G instrument for automation and subsequent deployment.

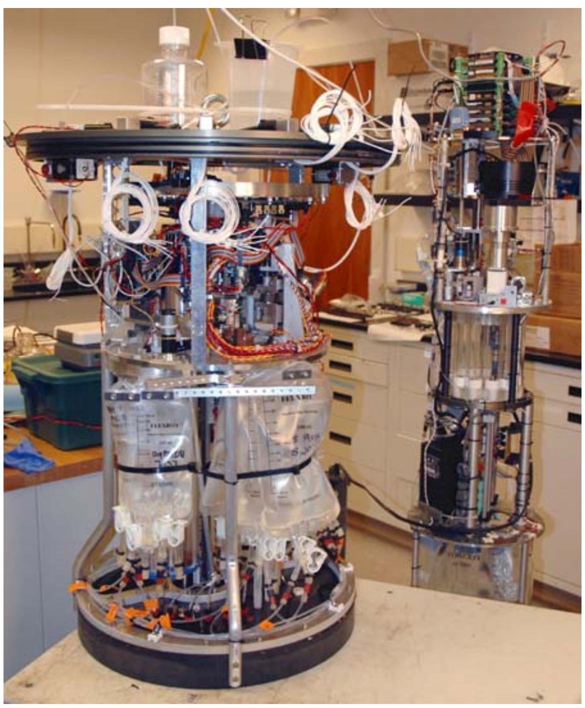

Figure 1. View of the 2G ESP as it nears completion (foreground, left) and undergoes testing in the laboratory. Its predecessor, the 1G ESP, stands in the background (right). Visible on the 2G device are many structural and electrical components, as well as bags of fluids that supply reagents and flush fluids. The 2G ESP is shown attached to the top bulkhead of its pressure housing. The eyebolt at the top is used for lifting the instrument and lowering into its pressure housing (not shown). The spools of white wire will connect external contextual sensors (e.g., CTD, fluorometer, transmissometer) to the ESP’s computer control and communication system. The black plastic base includes reservoirs for waste fluids. The overall size of the 2G ESP as shown here is approximately 24” (top bulkhead dia) x 36” (ht), as compared to 12” (dia) x 72” (ht) for the 1G ESP.

RESULTS TO DATE

The 2G ESP performs all of the analytical functions of its 1G predecessor with much greater speed, precision, and uses considerably less power. The process of collecting a sample employs a control algorithm that relies on pressure sensors above and below the filter medium to pace fluid pumping rate and prevent clogging. We discovered that rotary valves originally selected for this design leak at cold temperatures (e.g., <12^{o}C), warmer than the manufacturer’s specification. Valves used in the ESP must tolerate temperatures of near 0^oC, so the deployment of the new device has been delayed until this problem is rectified. Replacement valves that meet the cold temperature requirement have now been identified and tested. The 2G ESP will be fitted with these new valves (Jan ’06) and once that is completed the instrument will be ready for deployment in the ocean.

IMPACT AND APPLICATIONS

National Security

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

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 enable detection of 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, including input into predictive models aimed at forecasting events such as harmful algal blooms.

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 species’ life history stages, ecology/food webs, toxicology, bloom dynamics, physical/chemical oceanography, resource management, land use practices, policy making, and economics.

TRANSITIONS

Economic Development

Stanford University’s Office of Technology Licensing (OTL) assists MBARI in technology transfer and commercialization. The OTL has sent out information about the ESP, and in response to that solicitation SubChem Systems has executed a confidentiality agreement to learn more about the ESP and to assess opportunities for marketing the technology.

RELATED PROJECTS

A grant from the Moore Foundation has been awarded with the objective of enhancing the analytical capacities of the ESP, particularly in the realm of microbial environmental genomics (C. Scholin, PI). Under the auspices of a grant from the Keck Foundation (C. Scholin, PI), MBARI is building a prototype pressure housing and sample collection module suitable for deploying the 2G ESP in the deep-sea. This version of the instrument is known as the D-ESP. The goal of this project is to deploy the D-ESP in areas of intense geological and microbiological activity (e.g., hot vents, cold seeps) and link the spatio-temporal relationships between tectonics, fluid flow, and microbial flux from below the seafloor to overlaying waters using the same detection chemistries as those developed for surface water applications. As a step toward reaching that goal, the prototypical D-ESP will be mounted on an MBARI ROV so that it can be tested at variable depths and locations. Once that has been achieved, the D-ESP will be transitioned in 2006 to benthic observatories, including stand-alone moorings and cabled systems available at MBARI and elsewhere. Lastly, a grant from NASA (C. Scholin, PI; NNG06GB34G) was just awarded to continue the D-ESP work and conceptualize how a device of that kind may be applied to the search for life on other planets.

REFERENCES (*DELETE THIS SECTION IF THERE ARE NONE)*

Goffredi, S.K., W. Jones, C. Scholin, R. Marin, S. Hallam, R.C. Vrijenhoek. 2005. Molecular detection of marine larvae. *Marine Biotechnology* **8**: 1-12

Scholin, C.A., G.J. Doucette, and A.D. Cembella. In press. Prospects for developing automated systems for in situ detection of harmful algae and their toxins. In: Real-Time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Bloom, M. Babin, C.S. Roesler and J.J. Cullen, eds. UNESCO Publishing, Paris, France.

PUBLICATIONS

Scholin, C.A., G.J. Doucette, and A.D. Cembella. In press. Prospects for developing automated systems for in situ detection of harmful algae and their toxins. In: Real-Time Coastal Observing Systems for Ecosystem Dynamics and Harmful Algal Bloom, M. Babin, C.S. Roesler and J.J. Cullen, eds. UNESCO Publishing, Paris, France.