

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

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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 remotely, water-borne microorganisms and substances they produce. 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 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., Babin et al 2005, 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 toxins is emphasized. Collaborations with researchers outside of the core ESP project team make it possible to extend the detection capabilities of the instrument to include groups of marine bacteria and invertebrates (larvae).

APPROACH AND WORK PLAN

Technical Approach

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 and power consumption, and to take advantage of microfluidic-scale molecular detection technologies. Once the instrument is built, it will be tested in Monterey Bay.

Key individuals participating in this work - 2006

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.

Work plans for 2007

Specific plans for 2007 are as follows:

- 1) Deploy the 2G ESP in surface waters of Monterey Bay (Q2, 4). By Q4, deploy 2 instruments simultaneously.

- 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 3 copies of the 2G ESP to facilitate technology transfer
- 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)
- 5) Submit manuscripts and attend conferences/give presentations related to the ESP work completed.
- 6) In preparation for deep-water deployments, evaluate performance of the ESP when operated at 2°C.

WORK COMPLETED

The 2G ESP was fitted for surface water operations (Fig. 1), test operated to 10°C, and deployed several times in MBARI’s test tank and Monterey Bay (Fig. 2). Procedures for deploying and remotely operating the instrument were refined. All of the DNA probe array assays (bacteria, harmful algae, invertebrates) and an antibody-based assay for the algal toxin domoic acid (DA) were successfully ported to the instrument. Production of 3 copies of the instrument was initiated (completion expected mid 2007). Due to scheduling conflicts, the user’s workshop originally scheduled for late 2006 was deferred until late 2007.



Figure 1. The 2G ESP fitted to a structure for mooring the instrument below the ocean surface to 50m depth. The instrument is held in a pressure housing (center), which in this figure is cut away to show the ESP inside. The pressure housing is mounted on white, heavy plastic stand underneath which are 2, 12-volt rechargeable batteries designed for underwater use. A rotatable aluminum bail provides a connection via an electromechanical (EM) cable (top and left) between the ESP and a surface buoy fitted with a radio modem (not shown). To the EM cable are attached cream-colored oval shaped floats that support the cable as it rises to the surface expression. The entire assembly stands approximately 2 meters tall. Other sensors, such as those for temperature, salinity, depth, chlorophyll, etc., can be mounted to the ESP and their data transmitted ashore periodically.

RESULTS

The ESP has successfully automated application of 3 different classes of DNA probe arrays in single field deployments, targeting detection of marine planktonic organisms ranging from heterotrophic and photosynthetic bacteria, archaea and harmful algae, to small invertebrates found in the upper ocean. An antibody assay for DA, a neurotoxic amino acid, was also fielded in concert with the probe arrays. (http://www.mbari.org/microbial/esp/esp_technology.htm). Through application of species and toxin arrays, known toxic *Pseudo-nitzschia* spp. and domoic acid (DA) were detected concurrently onboard the ESP in Monterey Bay, CA. This is the first time that remote, integrated assessment of algal cell abundance and toxin concentration has been achieved in coastal waters.

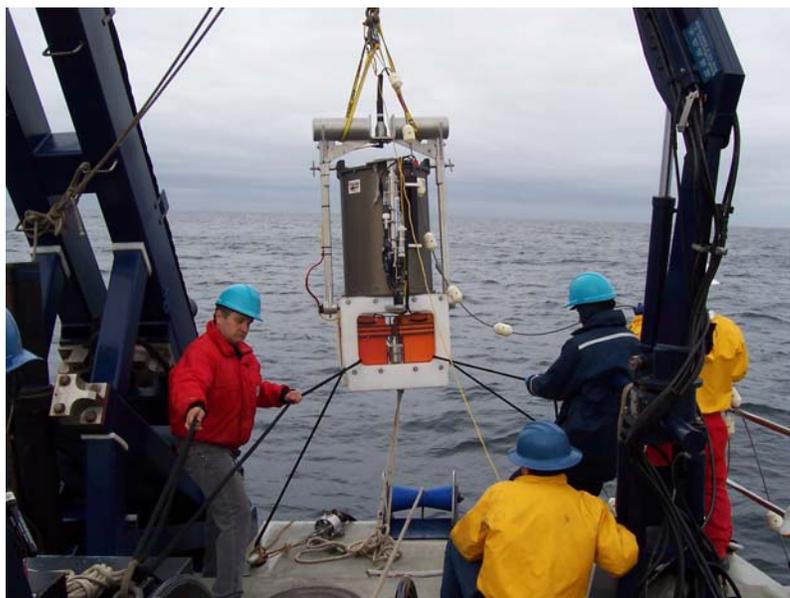


Figure 2. The 2G ESP being deployed in MBARI's test tank with divers inspecting the package (left), and the same instrument being deployed from the R/V *Zephyr* in Monterey Bay March 2006.

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, but to date no commercial partner has been identified. Recently, contacts with representatives at Agilent and Cepheid were established. Further discussions aimed at learning more about the ESP and possible business opportunities for marketing the technology are pending with both companies.

Science Education and Communication

The ESP website (<http://www.mbari.org/microbial/ESP>) is currently being updated. It is accessible from MBARI’s home site, which generates ~100,000 visitors monthly. Also, there is the additional ESP site (<http://www.mbari.org/esp>) that is nearing completion as well (a little more focused on the general public than the microbial/esp site). A news article for MBARI’s front page containing references to the ESP/ASTEP project was posted last March (<http://www.mbari.org/news/homepage/2006/esp-II.html>). Two animation sequences that illustrate function and deployment of the instrument (shallow, deep) are in production now. The animations will be incorporated into the MBARI ESP web site as well as the "Exploring Monterey Canyon" exhibit (http://www.mbayaq.org/efc/efc_mbh/dsc.asp?bhcp=1) that runs in the Monterey Bay Aquarium Auditorium daily (bringing in up to 1000 visitors/day). Two MBARI summer students (<http://www.mbari.org/education/internship/internpapers.htm>) have worked on this project to date. One was involved with development of the MARS web site (<http://www.mbari.org/mars/>) and D-ESP project pages (http://www.mbari.org/mars/general/deep_esp.html), while the other explored new approaches to printing DNA probe arrays for use with the ESP.

RELATED PROJECTS

A list of related projects is given at: <http://www.mbari.org/microbial/ESP/> (bottom of the page). 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 to 1000m. 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 overlying waters using the same detection chemistries as those developed for surface water applications. As a step toward reaching this 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 to benthic observatories, including stand-alone moorings and cabled systems available at MBARI and elsewhere. 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). A grant from NASA (C. Scholin, PI; NNG06GB34G) was 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. Lastly, the ESP project team recently joined a new NSF-sponsored Science and Technology Center, C-MORE (<http://cmore.soest.hawaii.edu/>). A thrust of C-MORE is to better understand how microorganisms mediate biogeochemical cycles in the global ocean, and to project how environmental change may affect microbial communities and the processes they mediate (http://cmore.soest.hawaii.edu/cmore_theme3.htm).

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