DETECTION OF WATER BORNE MICROBES USING AN AUTONOMOUS UNDERWATER SENSOR, THE ENVIRONMENTAL SAMPLE PROCESSOR (ESP)

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ABSTRACT

The advent of microfluidic techniques holds promise in the field of oceanography, particularly in the nascent field of ecogenomics, the study of genomes or gene products in microbial populations within the ocean. Despite this technical promise, a serious impediment lies in simply obtaining a sample to analyze. Solutions to this problem harken to the 19th century, namely ship-based expeditions to collect and filter water samples. While much sample processing can now occur on-board, more detailed analyses must wait until samples are returned to shore-side laboratories.

The Environmental Sample Processor (ESP) represents a first step towards addressing both the expense of sample collection and the time lag between sample collection, processing, and analysis. As a microbiology 'laboratory-in-a-can', the ESP performs all sample processing and analyses onboard using low-density DNA probe and protein arrays to assess in near real-time the presence and abundance of organisms, their genes and/or metabolites. In addition, a 2-channel real-time PCR module supports deployment of a variety of user-defined master mixes and primer/probe combinations. This arrangement provides assay flexibility, making *in situ* monitoring possible in a variety of environments. The ESP is battery operated but can also be attached to submarine cables and has been deployed in diverse oceanic environments for up to 45 days of autonomous operation.

KEYWORDS

In situ, water quality, robotic, molecular, autonomous, monitoring

INTRODUCTION

Increasingly, the application of microfluidics is making inroads into biomedical applications to help speed and simplify diagnoses. These 'sample-in, results-out' systems are impressive in their capabilities [e.g., 1,2], and are making exciting contributions to the world of personalized medicine. While these early successes are notable, the full application of microfluidic devices is still under development. Frequently, extensive peripheral instrumentation is required to prepare the sample, provide power and control, and perform analysis, thus increasing the size and complexity of the overall microfluidic device, making autonomous, unsupervised use quite difficult. Thus, current applications often benefit from well-provisioned laboratories with e.g., unlimited power and space for hardware, reagents, and waste.

While biomedical applications may represent the vanguard of microfluidic technology development, work is underway to apply microfluidic techniques in areas outside of medicine, where ISO certification standards may not apply, thus lowering barriers to development and application. Ecogenomic sensors, instruments that live in the environment of interest and autonomously collect and prepare a sample for molecular analyses, are ripe for microfluidic applications. For instance, continuous genetic analysis of microorganisms in the world's oceans opens a previously unknown window into the world of biogeochemical cycling and microbial ecosystem function. Sensors that exist within the environment of interest (i.e., *in situ*) also have advantages in water quality monitoring by reducing the time lag between detection of possible contaminants and notification of interested parties. Here we report on an instrument system called the Environmental Sample Processor (ESP) that represents a first step in the integration of microfluidic analytical methods into such an *in situ* sensor. Multiple successful field-deployments over the last 5 years illustrate the validity and usefulness of continuous monitoring using such an autonomous sensor.

Searching for specific organisms or metabolites in natural waters typically requires large sample volumes and the transport of material back to a laboratory for processing. These requirements necessitate transport of large volumes of water, and introduce delays ranging from many hours to days between material collection and analysis. The ESP is a field-deployable, robotic system that was developed to eliminate these limitations. The instrument combines autonomous sample collection capability with real-time molecular detection functionality allowing for remote detection of target organisms or their metabolites *in situ* (Fig. 1).

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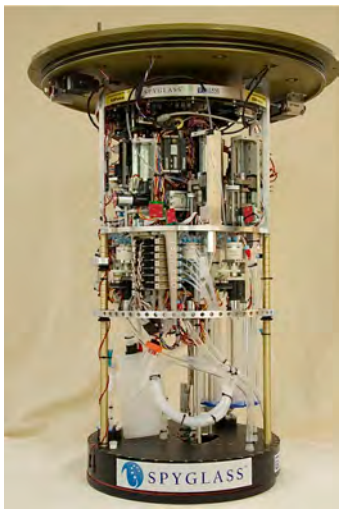
A.



B.

Figure 1. A. The ESP outside of its pressure housing. The instrument is approx. 1m tall and autonomously performs sample collection and analysis. B. Divers testing the ESP in a pressure housing within the MBARI test tank.

Currently, the ESP employs low-density DNA probe and protein arrays to assess in near real-time the presence and abundance of specific organisms, their genes and/or metabolites. In particular, we have developed a sandwich hybridization array that uses oligonucleotides to detect rRNA from a variety of harmful algae species including *Pseudo-nitzschia* spp., *Heterosigma akashiwo*, and *Alexandrium catenella* [3, 4]. In addition, a competitive ELISA has been developed to detect domoic acid, a phycotoxin produced by *Pseudo-nitzschia* spp. This toxin is an established threat to humans and wildlife [5] and high levels can have significant economic impacts on beach communities and aquaculture areas. For both these array types, results are generated within 3 hours, and returned to shore via radio or satellite link.

The unique sample collection and preparation capability of the ESP has fostered the development of new, more advanced 'back-ends' (called analytical modules) that permit molecular analysis beyond standard sandwich hybridization arrays. The first analytical module was developed in collaboration with Lawrence Livermore National Laboratory and consisted of a 2-channel quantitative PCR module attached to a microfluidics 'block' (MFB; Fig 2). The MFB successfully links the milli-fluidic world of the ESP and the micro-fluidic world of qPCR analysis with capabilities that allow low-volume measuring, mixing, and manipulation. The MFB supports deployment of a variety of user-defined master mixes, primer/probe combinations and control templates, and is the first device to successfully perform qPCR autonomously and remotely, literally within the ocean [6]. The development of the MFB identified the interface (i.e., fluidics, power, communications) between the sampling front end and the analytical back end as a critical component for integration of other analytical modules. Currently there is no standard for such an interface, but using the ESP as a model permits testing of various configurations for the most effective way to link sampling with microfluidic analysis.

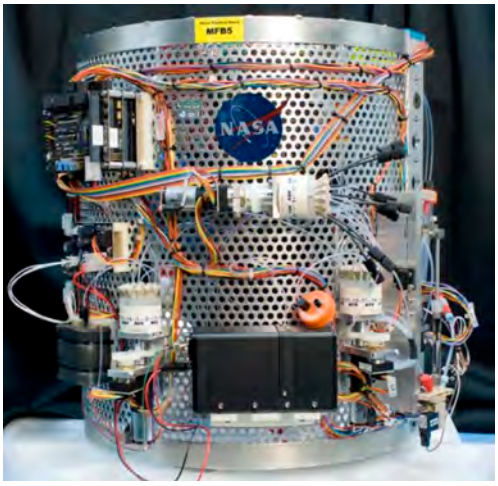


Figure 2. Microfluidic block (MFB) with attached qPCR module (black box). The MFB takes prepared sample from the ESP and performs qPCR using a variety of user-defined master mixes and primer/probe combinations.

An additional feature of the ESP is the ability to preserve samples for a variety of laboratory tests once the instrument is recovered. These archival samples can act as controls by corroborating the *in situ* results with traditional laboratory tests. As of yet, whole genome sequencing and metagenomic or metatranscriptomic analyses require shore-based laboratory equipment, and archived samples, collected over 30-45 days, enable these lab-based analyses.

Oftentimes goals over longer deployments change due to changing environmental conditions. Ideally, an autonomous sensor can be reprogrammed as warrented by changing environmental conditions. The ESP supports two-way communications for downloading new instructions so that its mode of operation can be altered from afar, and for transmitting results of autonomous analyses. This ability to communicate via radio, cell phone, or satellite phone has allowed the ESP to be deployed on a variety of platforms including coastal moorings, an open ocean drifter, a research ship, and a deep-sea "elevator" (Fig. 3). Most recently, it was used to conduct water quality assessments from a coastal mooring using sewage indicator microbes and harmful algal bloom species as primary targets. This presentation will highlight the architecture of the ESP and the microfluidic and analytical methods used onboard the instrument, results of recent field deployments, and an outline of plans for its further development.

Figure 3. The 'Deep-ESP' being readied for a 6-day deployment to the diffuse vent field of an underwater volcano (~1600m depth). This instrument performed sandwich hybridization arrays, archivals, and qPCR with only an occasional acoustic communications-link to the ship.

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