Conference paper

James Birch*

Collecting and processing samples in remote and dangerous places: the Environmental Sample Processor as a case study

https://doi.org/10.1515/pac-2018-0201

Abstract: Collecting water samples in remote or dangerous places can help identify chemical spills, discover clandestine weapons production, or determine if there has been natural or human-caused biological contamination of waterways. These collections can be expensive or put humans at risk due to the nature of the locale or the detection target. Such sample collection challenges are similar to those faced in oceanography, where accessibility and the physical realities of remoteness, corrosiveness, and pressure, place severe requirements on instrumentation, especially for unattended operations over long periods of time. The Monterey Bay Aquarium Research Institute (MBARI) has been at the forefront of developments that push forward sample collection and processing capabilities in the ocean. Specifically, the development of the environmental sample processor (ESP), a microbiology laboratory-in-a-can, has allowed extended presence with high frequency sampling. When deployed, the ESP filters water to collect particles, then either preserves those particles, or creates a homogenate for molecular analysis. Originally designed for detecting harmful algae blooms and the toxins they produce, the ESP now has expanded analytical capabilities. A newer version of the ESP is now being tested on an autonomous underwater vehicle, providing never-before-seen mobility and unprecedented access to the top 300 m of the ocean.

Keywords: Chemical Weapons Convention 2017; environmental monitoring; nucleic acids; sampling.

Introduction

Identifying chemical spills, chemical weapon production, or changes in the environment caused by such activities shares a fundamental requirement with oceanographic research. All work begins with acquiring sample material, and that work often requires material be gathered from places inaccessible or too dangerous to send humans. Additionally, in monitoring situations, samples must be gathered over long periods of time, greatly increasing the cost of collection. These are similar problems experienced within oceanography. Weather and sample location both impact where/when samples can be collected and, with ship-based expeditions, it is practically impossible to sample synoptically (i.e. many different locations at exactly the same time). Long-term sample collection at the same location is also difficult when expeditionary ship costs can approach or exceed $30 K/day.

Since its founding in 1987, the Monterey Bay Aquarium Research Institute (MBARI) has been developing technology to address these issues of access. Founded by David Packard (co-founder of Hewlett-Packard),

Article note: A special issue containing invited papers on Innovative Technologies for Chemical Security, based on work done within the framework of the Chemical Weapons Convention.

***Corresponding author: James Birch,** Director, SURF Center: Sensors, Underwater Research of the Future, Monterey Bay Aquarium Research Institute, Moss Landing, CA, USA, e-mail: jbirch@mbari.org

MBARI emphasizes the partnership between science and engineering to solve some of the most difficult problems in ocean sciences.

In particular, over the past 12 years we have been developing an instrument that would permit a more persistent, autonomous presence in order to either collect and preserve samples for return to the laboratory, or apply molecular analytical methods in situ. The challenges are many, as the self-contained instrument must collect and process samples autonomously with minimal communication to shore, and be deployed for 30–180 days. Our solution to these requirements is an instrument we call the environmental sample processor (ESP) and we have deployed versions of this instrument around the world since 2007.

The ESP is the first of a new class of instruments known as ecogenomic sensors. In oceanography, the term ecogenomic originally embodied the idea of merging high-throughput gene sequence data with environmental contextual data in order to better understand microbial ecology (i.e. who is present under what environmental conditions). Initially, this meant collecting and filtering water, and returning filters to a laboratory outfitted with the various technologies needed to perform genetic sequencing. Over time, this definition broadened to refer to sensor technologies that could apply molecular biology techniques to collected samples in situ. These sensors relied heavily on technologies from the medical diagnostics industry except now they were required to operate and survive in the environment of interest. Although this requirement demands a robust sensor technology, linking the sensor output with environmental parameters permits us to see direct connections between environmental parameters and genetic/molecular responses [e.g. 1, 2].

Despite the rapid advancement in both molecular biology techniques and instrument design, the implementation of the ecogenomic sensor concept has yet to reach its full potential. This is not due to a paucity of analytical methods, but rather the more prosaic difficulty of acquiring sample material. Whereas biomedical applications can utilize ml, ul, or even nl volumes when searching for targets, ocean work is often looking for molecules or organisms in tens of ml's or L's of water. Target scarcity results in 'sifting' through copious amounts of water to increase the chance of detection. Thus, most ship expeditions require large water processing stations, with the attendant bottles, peristaltic pumps, tubing, filter rigs and large numbers of personnel who filter water around the clock, often as quickly as it is collected.

This processing infrastructure can easily be carried and operated on ship-based expeditions. The precious filters can then be either processed in a portable laboratory onboard or returned to shore for more extensive analysis. The downside of this workflow is the same for any ship-based analysis; ship time is expensive, scientists can remain on site for only a limited time and when weather allows, and results are not usually available for at least 24 h once samples are returned to shore. These problems with ship-based science drove Chris Scholin of MBARI to envision a robotic device that could 'take the laboratory to the sea', remain there for long periods of time, provide results to shore in near real-time, and mitigate the risk associated with sending humans to sea.

The ESP

The 'second generation' ESP (2G-ESP) is a successful iteration of this robotic concept. The 2G-ESP is a modular electro-mechanical system that allows a wide range of capabilities depending on the deployment scenario or science question being asked (Fig. 1).

Typically moored in the sea, the battery-powered 2G-ESP is attached to an anchor via a taut-line and samples water through openings in the top of the pressure-housing. A flexible electro-mechanical cable connects the pressure housing to a small surface buoy that contains a cell phone modem, allowing real-time communications with the undersea instrument (Fig. 2). With low power consumption a major requirement in electronics design and judicious power usage accomplished through software control, we have been able to deploy the 2G-ESP in this mooring configuration for up to 45 days on 360 'D' cell alkaline batteris.

We have modified the pressure housing and buoy structure to allow other mooring configurations, which have resulted in the 2G-ESP operating in water from 800 m to over 1800 m deep [3, 4], or feely drifting to repeatedly sample the same water parcel [5].

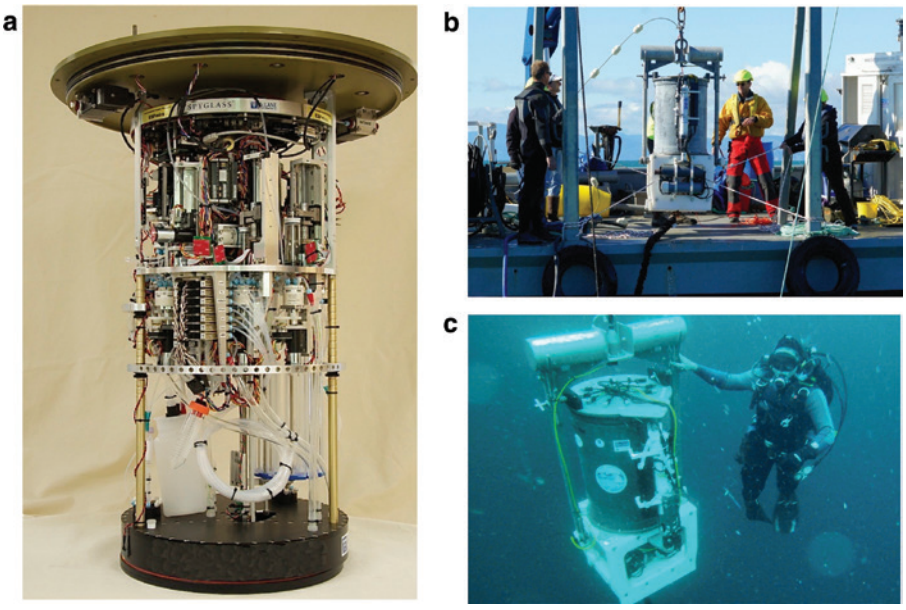

Fig. 1: The Environmental Sample Processor (ESP) is a self-contained, autonomous, robotic water sampler capable of performing several types of molecular biological assay in situ. (a) Instrument attached to pressure housing lid. (b) Deploying the ESP in a New Zealand shellfish farm. (c) ESP in surface mooring configuration with diver support.

Regardless of the mooring, there are multiple types of assays the 2G-ESP can perform. The original technique was based on a technique known as the Sandwich Hybridization Assay (SHA). SHA probe arrays detect ribosomal ribonucleic acid (rRNA) sequences through a chemiluminescent reaction [6], and allow the identification of microorganisms as well as estimates of their density. In addition to SHA, a competitive ELISA assay has been developed for the detection of non-nucleic acid targets (e.g. proteins), which has proven useful in detecting harmful algae toxin, often present in areas affected by red tide and harmful algae blooms. For more detailed explanations of the assay techniques used in the ESP, see e.g. [7–9].

The SHA and competitive ELISA are tests that work well with large amounts of biomass, but are less sensitive to rare targets. Adding quantitative PCR (qPCR), a highly specific and sensitive technique for identifying rare targets, required modifications to the core 2G-ESP instrument such that uL's of fluid could be manipulated precisely and repeatedly. Thus, we created a separate fluid handling system dubbed the "microfluidic block", or MFB that can be attached to the core instrument via single electrical and fluidic connections. A 2G-ESP with an MFB and qPCR capability has been deployed on coastal moorings [10, 11] and later on an open-ocean drifter [5], revealing surprising variability in microbial community structure and function over short time (<32 h) and small spatial scales (<30 km).

Another capability of the 2G-ESP is the ability to preserve samples for more in-depth analysis once the instrument is recovered and returned to the laboratory. This process begins with filtering seawater and collecting sample, then applying a preservative and storing the filter until instrument recovery. This ability to return preserved samples allows collection of multiple samples over several-month durations, allowing investigation of daily, weekly, or seasonal variations in organism abundance or gene expression. Currently the gene targets of the 2G-ESP range from archeal, bacteria, diatoms, dinoflagellates, water quality indicators, various invasive species, and environmental DNA ranging from bacteria to whales.

The future: portability and mobility

Despite the success of the 2-G ESP, the system is still large, bulky and heavy, requiring medium/large ship capabilities, and sometimes divers to complete the deployment/recovery. Additionally, an inherent shortcoming of moored systems is that they sit in one location waiting for targets-of-interest to drift by. Thus, moorings

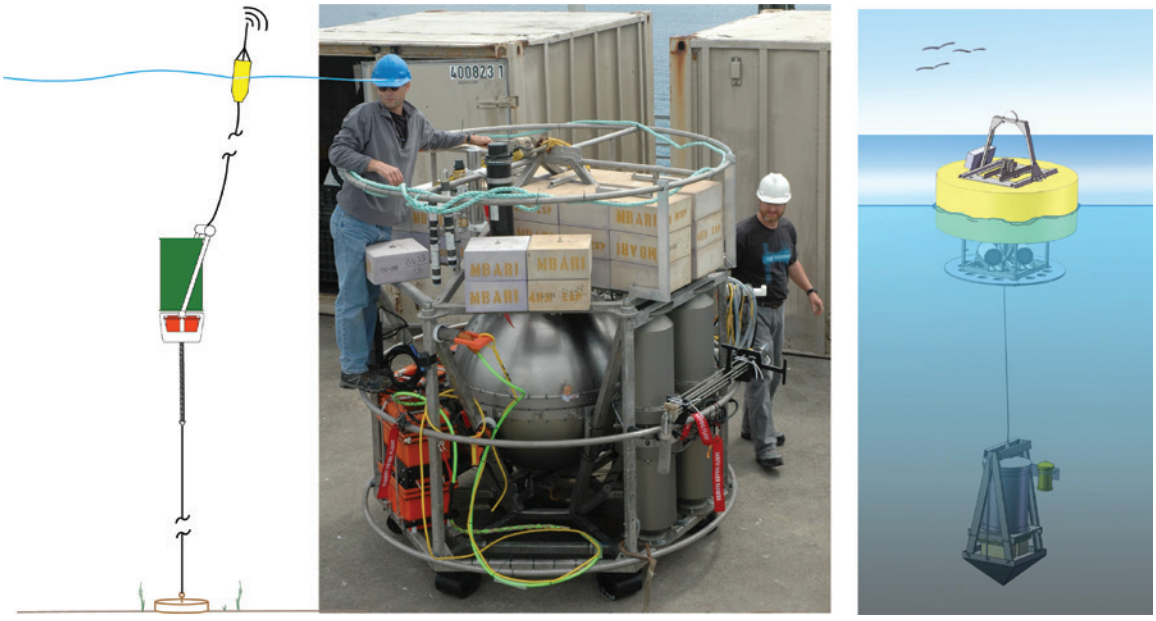

Fig. 2: Different mooring configurations allow access to different environments. A typical moored configuration (left) places the 2G-ESP up to 50 m deep, with communications via a buoy cell phone. A 1 m titanium sphere allows deployments to depths of 4000 m (middle) and communication via acoustic modem. A freely drifting configuration (right) can remain in the same parcel of (moving) water and collect samples over time.

are inherently measuring events that began 'upstream' of where samples were collected; these results can be difficult to interpret if we want to know what environmental variables might turn certain genes on/off. Thus, while a more persistent presence is clearly advantageous over periodic ship expeditions, the ability to move and query regions of the environment as that environment changes would provide distinct advantages in understanding the links between physical measurements and biological responses of microorganisms.

Over the last 4 years, we have thus focused our efforts on the development of a new generation of ESP instrument, one that is smaller, more modular, and less expensive than the 2G-ESP. This instrument, called the 3G-ESP (for 'third generation') had the design requirement that it must fit in the payload section of a long-range autonomous underwater vehicle (LRAUV), previously developed at MBARI (Fig. 3). This requirement has focused development on small size, but the instrument itself is agnostic to the platform it is connected to, and a hand-carried version for water-quality work is being developed.

Fig. 3: The long-range autonomous underwater vehicle (LRAUV) with the 3G-ESP mounted in the forward yellow body section.

In order to meet the goal of smaller size, the 3G-ESP has been designed around the concept of a processing cartridge, which carries filter material, processing reagents, power and logic, all arranged to fit within a 12″ diameter cylinder (Fig. 4). These cartridges are self-contained, and can be selected individually depending on sampling requirements. The 3G-ESP has been designed for 60 unique sampling events, which can then be archived or processed, depending on the science questions being asked. Two cartridge types exist, one for sample preservation and another for sample lysis and handoff for downstream processing. Sample preservation requires filtration and application of a preservative, similar to preservation in the 2G-ESP. We have run experiments to show that nucleic acids (DNA and RNA) can be preserved with high fidelity even after 8 months storage in cartridges held at room temperature [unpublished data and 12]. The second cartridge type (the 'lysis' cartridge) applies a lytic agent and heat to the filter, creating a homogenate of nucleic acids, proteins, etc. that will be the raw material for more in-depth processing downstream via an analytical module.

Analytical modules can be of many types as long as they can fit in the payload space provided, and meet the power and communications requirements of the vehicle. One module we have used employs the method of Surface Plasmon Resonance (SPR) [13–15], a technique that allows us to test for the toxin domoic acid, commonly produced by diatoms of the genus *Pseudo-nitzschia*. Other modules under development can perform digital droplet PCR (ddPCR) or Total Internal Reflection Fluorescence (TIRF) on the homogenate. Each analytical method has its unique strength and weakness; their shared strength is the ability to utilize raw homogenate from the cartridge. In the future, in addition to filtering particulates from water, we plan to

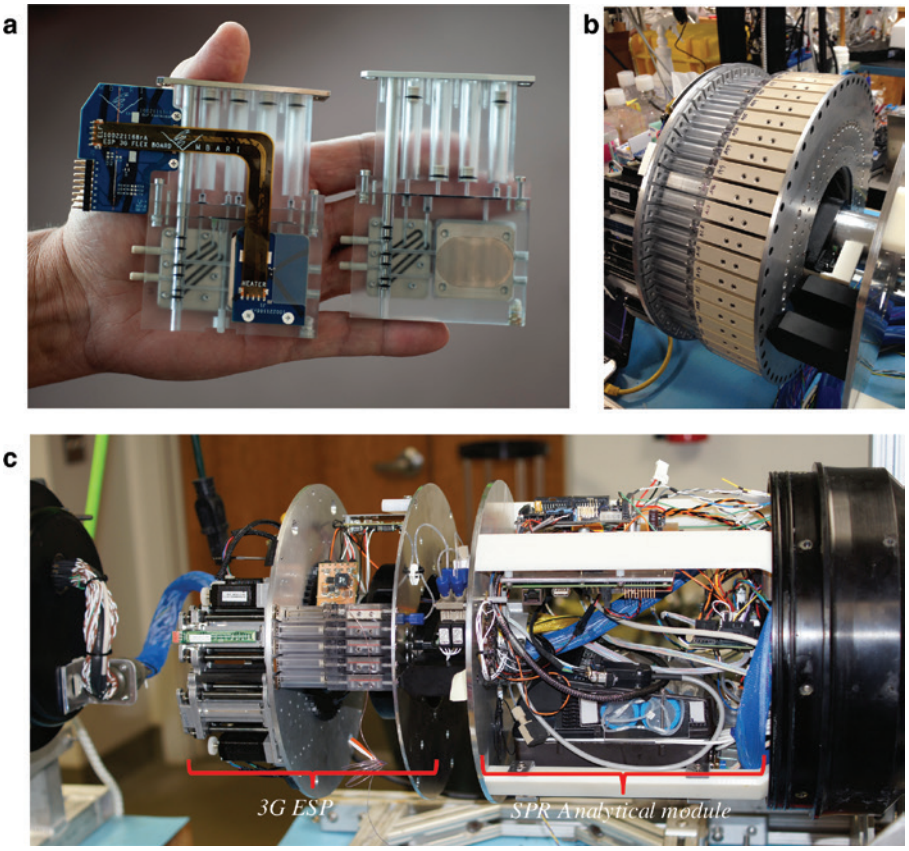

Fig. 4: (a) The 3-G ESP cartridges are of two varieties capable of lysis with integrated heater (left), and archival (right). (b) Sixty cartridges fit onto a circular valve assembly that is fitted into a 30 cm diameter by 71 cm housing in the forward space of the LRAUV. (c) The complete payload section with five cartridges loaded. Forward of the cartridges is space for analytical modules, instruments capable of processing the homogenate created in the cartridges. In this iteration, we tested a surface plasmon resonance (SPR) instrument useful for harmful algae toxin detection.

design a cartridge that contains a sorbent material to capture dissolved substances. These substances could then be eluted off the cartridge and passed 'downstream' to a connected mass spectrometer for identification of chemical species without foreknowledge of what might be present.

Conclusions

The ocean can be a difficult and dangerous environment in which to work, whether attempting a one-time sampling event or remaining for a prolonged deployment. Technology can mitigate much of this risk; at MBARI we have developed one solution for acquiring sample in such an environment – a robotic device known as the ESP that effectively brings the laboratory to the sea. Iteration on the original ESP design has resulted in a smaller, more modular sampling system that can be carried in terrestrial environments, or mounted on underwater vehicles providing unfettered mobility in the upper 300 m of the ocean. We feel this new capability will transform sample collection and subsequently our view into ecological processes within the ocean. This ability also presents exciting new opportunities in the field of multi-vehicle control, adaptive sampling, and artificial intelligence. As an initial test of multi-cartridge sampling, we have performed several flights to test different vehicle behaviors and/or sampling regimes [16]. The success of these initial test flights is exciting and points to a future where water sampling can be performed autonomously, triggered on any number of contextual measurements (e.g. chlorophyll, temperature, salinity, depth) depending on the science question asked.

References

[1] C. Scholin. *Ocean Sci*. **6**, 51 (2010).
[2] C. Scholin. In *Encyclopedia of Biodiversity, Vol 2*, S. A. Levin (Ed.), pp. 690–700. Academic Press, Waltham, MA (2013).
[3] W. Ussler, C. Preston, P. Tavormina, D. Pargett, S. Jensen, B. Roman, R. Marin III, S. Shah, P. Girguis, J. Birch, V. Orphan, C. Scholin. *Env. Sci. Tech*. **47**, 9339 (2013).
[4] H. Olins, D. Rogers, C. Preston, W. Ussler III, D. Pargett, S. Jensen, B. Roman, J. Birch, C. Scholin, M. Haroon, P. Girguis. *Front. Microbiol*. **8**, 1042 (2017).
[5] J. Robidart, M. Church, J. Ryan, F. Ascani, S. Wilson, D. Bombar, R. Marin III, K. Richards, D. Karl, C. Scholin, J. Zehr. *ISME J*. **8**, 1175 (2014).
[6] C. Scholin, K. Buck, T. Britschgi, J. Cangelosi, F. Chavez. *Phycologia* **35**, 190 (1996).
[7] C. Preston, R. Marin III, S. Jenson, J. Feldman, J. Birch, E. Massion, E. DeLong, M. Suzuki, K. Wheeler, C. Scholin. *Env. Microbiology* **11**, 1168 (2009).
[8] C. Scholin, G. Doucette, S. Jensen, B. Roman, D. Pargett, R. Marin III, C. Preston, W. Jones, J. Feldman, C. Everlove, A. Harris, N. Alvarado, E. Massion, J. Birch, D. Greenfield, R. Vrijenhoek, C. Mikulski, K. Jones. *Oceanography* **22**, 158 (2009).
[9] G. Doucette, C. Mikulski, K. Jones, K. King, D. Greenfield, R. Marin III, S. Jensen, B. Roman, C. Elliott, C. Scholin. *Harmful Algae* **8**, 880 (2009).
[10] C. Preston, A. Harris, J. Ryan, B. Roman, R. Marin III, S. Jensen, C. Everlove, J. Birch, J. Dzenitis, D. Pargett, M. Adachi, K. Turk, J. Zehr, C. Scholin. *PLoS One* **6**, e22522 (2011).
[11] J. Robidart, C. Preston, R. Paerl, K. Turk, A. Mosier, C. Francis, C. Scholin, J. Zehr. *ISME J*. **6**, 513 (2012).
[12] E. Ottesen, R. Marin III, C. Preston, C. Young, J. Ryan, C. Scholin, E. DeLong. *ISME J*. **5**, 1881 (2011).
[13] J. Mitchell. *Sensors* **10**, 7323 (2010).
[14] T. Chinowsky, J. Quinn, D. Bartholomew, R. Kaiser, J. Elkind. *Sens. Actuators B* **91**, 266 (2003).
[15] R. Stevens, S. D. Soelberg, B. Eberhart, S. Spencer, J. Wekell, T. Chinowsky, V. Trainer, C. Furlong. *Harmful Algae* **6**, 166 (2007).
[16] Y. Zhang, B. Kieft, R. McEwen, M. Stanway, J. Bellingham, J. Ryan, B. Hobson, D. Pargett, J. Birch, C. Scholin. *MTS/IEEE Oceans'15* doi:10.23919/OCEANS.2015.7401969 (2015).