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THE QUEST TO DEVELOP ECOGENOMIC SENSORS

A 25-Year History of the Environmental Sample Processor (ESP) as a Case Study

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> For the past 25 years, MBARI has worked to develop an instrument that exemplifies the ecogenomic sensor idea, proving its viability, commercializing the first working example of the device, and providing support for its operation at various locations around the world.

ABSTRACT. Roughly 25 years ago, "ecogenomic sensors" were conceived of as autonomous devices that would be used to apply molecular analytical techniques below the sea surface as one part of a futuristic, integrated ocean observing system. The Environmental Sample Processor (ESP) was built to address that idea—an instrument to help define both the technological and operational elements that underlie the ecogenomic sensor concept. Over time, the ESP emerged as a working example of that class of instrument, enabling the application of DNA probe and protein arrays as well as use of the quantitative polymerase chain reaction (qPCR) technique to assess the presence and abundance of a wide range of organisms, specific genes, and metabolites. The ESP is also used to preserve samples for a variety of laboratory tests not yet possible to carry out in situ (e.g., DNA sequencing). The instrument has been deployed on a variety of platforms, including coastal moorings, piers, an open ocean drifter, research vessels, a shallow water benthic lander, and a 4,000 m rated "elevator" designed for use on deep-sea cabled observatories. A new version of the ESP is currently being developed for use aboard an autonomous underwater vehicle. This article traces the evolution of the ESP from its conception to present-day status.

INTRODUCTION

When David Packard founded the Monterey Bay Aquarium Research Institute (MBARI) in 1987, he foresaw endless opportunities for inventing and utilizing new ocean sensor technologies. His vision was broad and unencumbered, and he challenged scientists and engineers to look beyond the horizon to realize new capabilities (Packard, 1989). Sensors for characterizing a variety of chemical, physical, and optical properties of the water column were evolving rapidly, as were the platforms for fielding them (see Brewer et al., Chavez et al., Sakamoto et al., and Robison et al., 2017, all in this issue). Collectively, these developments heralded a new era of ocean science—one in which measurements could be acquired instantaneously and remotely in ways not previously possible.

At that time, the biomedical research and diagnostics industries were advancing rapidly. The emergent tools and techniques coming from those domains catalyzed many groundbreaking innovations for observing microbial life in the sea (e.g., Pace et al., 1986; Chisholm et al., 1988). Nucleic acid sequencing and the application of DNA and antibody probes were quickly proving to be powerful tools for revealing the diversity and abundance of a host of species (e.g., Olsen et al., 1986; Giovannoni et al., 1988, 1990; DeLong et al., 1989; Amann et al. 1990; Schmidt et al., 1991). Despite the growing use of molecular analytical methods in the ocean sciences, those approaches were constrained by the need to return samples to a laboratory for processing; applying such techniques in the field, "on the fly," was largely impractical. That limitation, combined with Packard's charge for innovation, inspired an idea to create an instrument that would fully automate the steps of sample collection and handling so that routine molecular analyses could be carried out autonomously within the environment of interest, below the sea surface.

When the Environmental Sample Processor (ESP) was first conceptualized in the early 1990s, no device of its kind existed, and the prospects for its realization seemed farfetched. Yet, the drive to develop point-of-care devices for medical applications suggested that such a development was possible. How could we leverage cutting-edge advances in biomedical diagnostics to enable the utilization of molecular probe technology at sea and in real time? Addressing that question required solving a number of interrelated problems, such as devising and packaging analytical chemistries for use outside of a laboratory, pairing those methodologies with appropriate fluid manipulation mechanisms, and finding a means to capture and sequester samples for

FACING PAGE. MBARI mechanical engineer Brett Hobson (left) and Research Specialist Chris Preston after recovering the Long-Range AUV *Makai* during sea trails of the newest version of the Environmental Sample Processor (ESP). Preston's left hand rests on the payload section where the ESP resides. *Image credit: Brian Kieft*

processing. Developing the ESP required a coordinated science/engineering effort, a challenge well matched to Packard's overarching vision.

Over the ensuing decade, the concept of an ESP-like device slowly gained in popularity (Scholin et al., 2001; Babin et al., 2005). With that optimism came a growing body of literature and artistic renderings that aimed to further define the idea (e.g., Paul et al., 2007). A particularly influential image came in 2005 from Hunter Hadaway, a graphic artist at the University of Washington's Center for Environmental Visualization, highlighting what were dubbed "ecogenomic sensors" embedded within a futuristic ocean observing system (Figure 1). Prior to that time, the term "ecogenomics" was being used to describe the merger of DNA sequencing with environmental science. Among the many attempts to capture this idea graphically was a figure promoted by Anna Palmisano, who combined images of a satellite, a ship, and a DNA probe array to illustrate how genomic approaches for studying marine microbes could be combined with other ocean observing capabilities. University of Washington planktonologist E. Virginia Armbrust encouraged Hadaway to meld Palmisano's ideas with the notion of an ocean bottom cabled observatory and satellite imagery, leading to the image shown in Figure 1.

Hadaway's illustration cleverly captured the essence of what an ocean-going molecular biological sensor might encompass, and simultaneously gave it a name. Importantly, the device was placed within a distributed sensory network that could capture the environmental setting on scales ranging from satellite to microscopic imagery, all framed within a dynamic chemical and physical context. A holistic, integrated view of the prevailing environment would be necessary for interpreting ecogenomic sensor measurements. The image solidified the concept and generic name of this emergent class of instrument, and spoke to the importance of its being one part of a larger, integrated system. However, developing a functioning example of an ecogenomic sensor proved far more difficult than was first imagined (Scholin, 2013). This paper summarizes some of the challenges we faced while developing the ESP, reviews progress made using the device in a range of oceanographic settings, reflects on aspects of the design that have

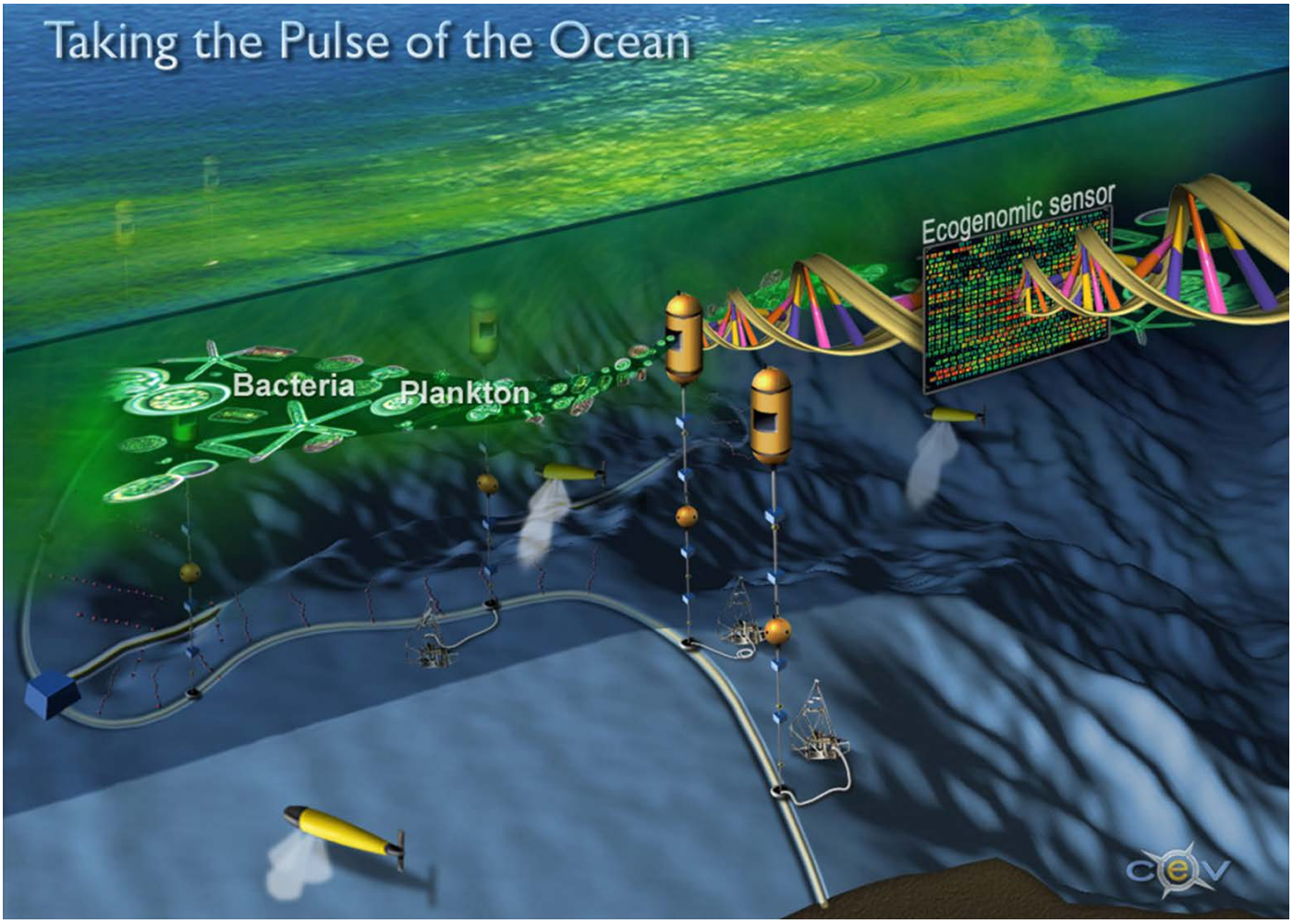

FIGURE 1. Artist's rendition of "ecogenomic sensors" (center) embedded within an array of other ocean sensors. The image implies that bacteria and plankton are drawn into the device and their nucleic acids analyzed using an array of probes to detect a variety of specific genes and gene products. *Figure courtesy of E. Virginia Armbrust and the Center for Environmental Visualization at the University of Washington Seattle*

proven successful as well as limiting, and outlines the present-day effort to reinvent the device for use aboard autonomous underwater vehicles (AUVs).

HISTORY OF THE ESP CONCEPT

The motivation for developing the ESP initially grew from studies of harmful algal blooms (HABs). Traditionally, such investigations required transporting discrete field samples to a laboratory, where microscopy was used to identify and enumerate particular organisms. This process could take days or longer to complete, which prompted the development of rapid methods for identifying and quantifying species of public health concern (e.g., Scholin and Anderson, 1998). It was particularly important to distinguish between morphologically similar toxic and nontoxic HAB species, and so species-specific molecular probes were developed to augment microscopy-based cell counting methods (Scholin et al., 2003). Nevertheless, in the early 1990s, the time it took to process samples using then state-of-the-art molecular probe detection techniques was still orders of magnitude longer than making many physical, chemical, or optical measurements at sea using commercially available sensors.

Creating a portable "molecular biology laboratory in a can" was one solution to reducing the time interval from sample collection to HAB warning. Apart from instrument design, a major hurdle was overcoming the conventional wisdom derived from decades of traditional laboratory practices. For instance, molecular analytical methods rely on a suite of perishable biochemical reagents, all of which are typically maintained and utilized under strict regimes. Laboratory equipment used throughout the different steps of analyses benefits from a clean environment, ample working space, and an essentially endless supply of electricity. Translating the use of such laboratory-based techniques to an autonomous machine deployed for extended periods underwater demanded inventing and adopting an entirely different approach to reagent formulation, storage, and use. This analytical workflow and the required expendable supplies had to be compatible with hands-off operation, ambient field conditions, minimal battery power, and the materials comprising the instrument itself. Hence, much of the early work developing the ESP was focused on assay chemistry and workflow refinement as well as defining the accompanying engineering specifications for every aspect of operation, from water intake to data transmission.

It quickly became clear that another major technical difficulty would be enabling fluid handling over six orders of volumetric magnitude. More often than not, it would be necessary to concentrate particles from liter or multi-liter quantities of native water prior to initiating further sample processing. Analytical schemes that followed would generally require applying a series of reagents to the concentrated material on milli- and micro-fluidic (mL, μL) scales. In all, the ESP would have to handle "live water" that contained an ever-changing, diverse assemblage of organisms and particulate matter, as well as highly refined, sterile, and sensitive biochemical reagents. Native water and analytical reagents would have to be partitioned within the instrument and the integrity of each not compromised as the device repeatedly handled multiple samples. What could possibly go wrong? These engineering specifications pointed to an instrument of much greater complexity and nuance than had been popularized artistically.

FROM CONCEPTUALIZATION TO WORKING INSTRUMENT

At the time the ESP project was conceived in the early 1990s, sequencing of ribosomal RNA (rRNA) genes was widely employed for assessing the phylogenetic relationships of organisms (e.g., Field et al., 1988). Comparing different sequences revealed unique "signatures"—short stretches of genetic code—that could be used to identify particular organisms. Methods for detecting those unique sequences were well established using both whole-cell and cell-free methods (e.g., Scholin and Anderson, 1994; Scholin et al., 1994). Whole-cell assays employed species-specific, fluorescently labeled, rRNA-targeted DNA probes to visualize individual cells (e.g., DeLong et al., 1989). In contrast, the cell-free technique relied on homogenizing cells and extracting nucleic acids; the extract was fixed to a membrane and the presence/abundance of particular sequences was revealed using radioactive or colorimetric DNA probes (e.g., DeLong, 1992; Dyson, 1991). Scholin et al. (1996) advanced those complementary approaches to refine rapid detection methods for specific HAB species as the analytical foundations for developing the ESP. Conveniently, the sample acquisition and handling workflows of these methods used very similar, and repeated, sample-processing techniques. What differed was how the assay end result was interpreted: the whole-cell technique rested on enumerating fluorescently labeled intact cells, whereas the cell-free techniques employed a colorimetric scheme that was responsive to the abundance of target molecules found in a sample homogenate.

Because different rRNA detection methods had similar requirements prior to the actual assay end result, it seemed feasible that we could develop a single robotic device that would enable multiple types of sample handling procedures. Such a device would need to facilitate individualized sample processing, a requirement that ultimately gave rise to the idea of a "puck" (Figure 2). Different types of pucks could be used to capture samples and contain them throughout various stages of processing, but all would conform to standard external dimensions. In this way, a robotic mechanism could select pucks from a cache and move them into and out of one or more fluid handling stations for carrying out distinct steps of sample concentration, chemical processing, and imaging (Roman et al., 2007; Scholin et al., 2009). The idea of

using pucks was a first step toward making ecogenomic sensors modular; a set of pucks within the instrument could be used to accommodate different sample handling functions so long as manipulation of those individualized chambers were within the capabilities of a common robotic device.

Miller and Scholin (1998) prototyped the ESP puck idea using a custom made filtration manifold for concentrating samples, preserving them, and applying reagents to reveal HAB species using the whole-cell detection method. This technique was very simple and effective, and automating the steps associated with that procedure was relatively straightforward. The difficulty came in autonomously capturing the resulting signal, because it required very-high-resolution epifluorescence imaging of a filter's surface. Even more challenging was the number of individual species that could be distinguished in a single sample given limited fluorescence excitation/emission spectra of available reporting dyes.

Consequently, we turned our attention to developing cell-free detection methods with the objective of simultaneously detecting many target species in a single sample using an ordered array of species-specific probes, analogous to what the ecogenomic sensor illustration implied (Figure 1; Scholin et al., 1999). We reasoned that the sample preservation scheme used in the whole-cell identification method (Miller and Scholin, 2000) could provide a means to stabilize material collected by the ESP over the course of a deployment. Once the instrument was recovered, the preserved samples could be subjected to whole-cell labeling as a way to verify results obtained remotely and in real time using the probe arrays.

The notion of using an ordered array of different probes to detect a variety of organisms in a single sample was very appealing, but the established methods used widely in the 1980s and 1990s by those in the oceanographic community (e.g., Delong, 1992) would not easily work in the context of the ESP. For example, the protocols involved a prolonged series of incubation and wash steps, used radioactive isotopes, and employed x-ray film as the primary medium for recording results of the tests. MicroProbe, a biotechnology company, offered an elegant alternative that sought to use rRNA as a basis for detecting infectious organisms. The goal was to enable sample processing in a patient/doctor clinical setting so that an appropriate course of treatment could be prescribed in a single, one-hour meeting. A novel sandwich hybridization assay (SHA) made that possible. Sample material was concentrated, exposed to a lytic reagent, and heated, and the resulting crude (i.e., unpurified) homogenate was used directly at room temperature in a semi-automated, colorimetric-based target sequence detection scheme (Van Ness and Chen, 1991; Van Ness et al., 1991). Like many other advances in biomedical diagnostics, MicroProbe's clever assay chemistry offered a new insight for how we might rapidly detect specific marine microorganisms both in the laboratory and in an ocean-deployable instrument. Scholin et al. (1996, 1999) partnered with MicroProbe (later renamed Saigene) to adapt the SHA technique for HAB species detection, work that ultimately culminated in a test system that is still in use to this day (Marin and Scholin, 2010).

In order for the SHA method to have utility in the ESP, it was necessary to

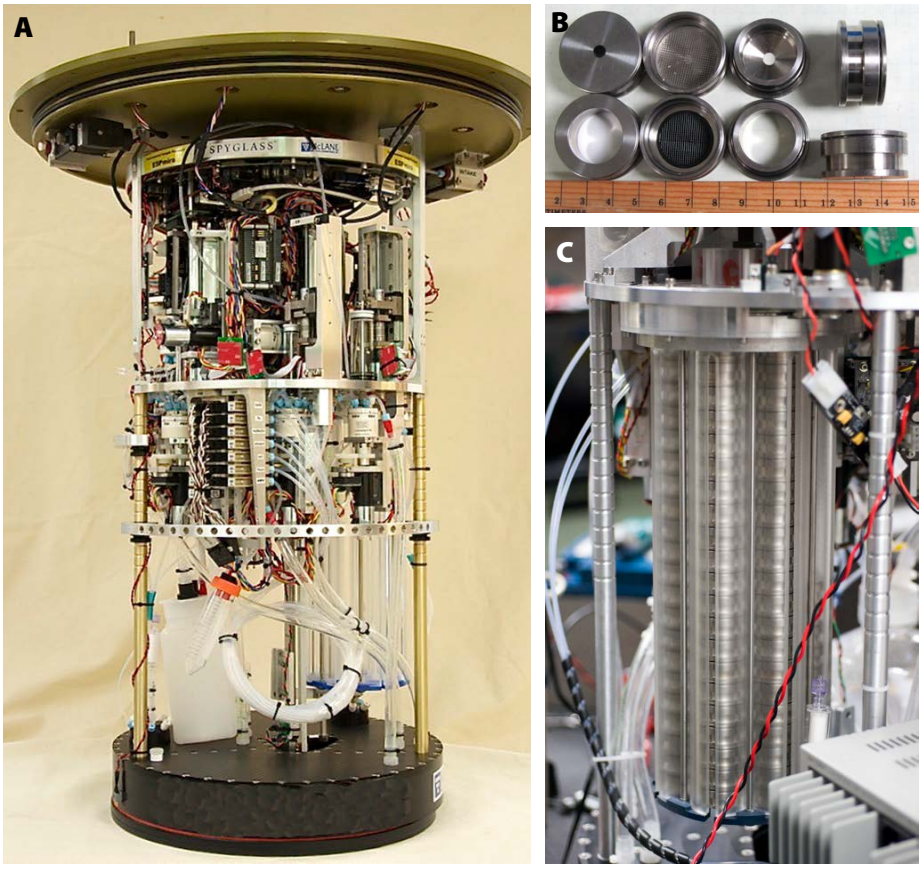

FIGURE 2. (A) The present-day Environmental Sample Processor (ESP) and (B) "pucks" used for sample processing (Scholin et al., 2009). The bulk of the instrument's electromechanical systems are on the top half of the device. (C) A portion of the rotating carousel that holds the pucks is visible on the mid-lower right side at the back of the ESP. By accessing a set of pucks configured with a variety of filters or chemically reactive media and using them in concert with directed fluid movements, the ESP can perform a variety of sample handling and analytical schemes remotely over the course of a single deployment. The hollow black base captures waste fluids. Reagents used for sample processing (not shown) are attached to the frame. The ESP is commercially available from McLane Research Laboratories Inc. (East Falmouth, MA). The ESP is ~0.5 m in diameter and 1 m tall. The instrument is protected under US Patent No. 6187530.

modify the chemistry so that it worked in a filter-based array format (Figure 3). By replacing the colorimetric detection method originally employed for clinical diagnostic applications with one based on chemiluminescence, we discovered that a digital camera could be used to passively capture an image of the light emitted from an array printed on a porous filter. The end result was similar to what is obtained using the traditional array methodology, except that the process was much faster, used no radioactive materials, culminated in a digital record, and was amenable to automation. Aside from contextual environmental measurements (e.g., temperature, salinity), real-time data obtained from the ESP thus depended on transmitting only an image file. By using image analysis software to measure the intensity of light associated with each probe-sensing element on the array, we could ascertain whether a particular organism or group of organisms was present in a sample and use that signal to derive estimates of their abundance. The feasibility of using this approach to detect HAB species remotely, below the sea surface, was first demonstrated in 2001 (Babin et al., 2005; Scholin et al., 2008). Though the probe arrays were very crude at the time, there was no doubt that the mechanical and biochemical approach adopted for remotely detecting rRNA sequences worked. This was the first time that DNA probe technology had been fully automated for use aboard a submerged, battery-operated ocean sensor.

With the SHA sample collection and handling methodology in place, array printing was rapidly improved using contact pin and piezoelectric contactless "probe ink" deposition techniques (Greenfield et al., 2006, 2008; Figure 3). The SHA probe repertoire was expanded to identify different groups of bacteria, archaea, microalgae, and invertebrates (e.g., Tyrrell et al., 2001; Goffredi et al., 2006; Jones et al., 2007; Haywood et al., 2007; Greenfield et al., 2008, Mikulski et al., 2008; Preston et al., 2009). Reagent composition evolved so that all-liquid solutions needed for the SHA and whole-cell preservation could be stored in flexible bags or other sterile containers for many months over temperatures ranging from ~4°C to ~30°C. Eventually, the entire SHA process, from collection of a live sample to transmission of an array image to shore, came to take approximately two hours. Greenfield et al. (2006, 2008) then demonstrated how estimates of the abundance of target species obtained in situ using the SHA arrays could be validated post deployment using the whole-cell probing technique.

Although the SHA worked well, its application was limited to detecting rRNA; potential users of the ESP also wanted to detect a variety of other biomolecules, such as toxins. In such cases, antibody and receptor-based detection techniques were available—the question was how to translate their use to the ESP. Doucette et al. (2009) were the first to solve that problem. Instead of printing DNA probes onto filters, they devised a way to print an array of proteins that were then utilized as sensing elements in an analogous fashion to the SHA probes. Moreover, it was possible to run both the SHA and protein array assays sequentially on the same instrument. That capability was first applied to detect specific species of *Pseudo-nitzschia* (pennate diatoms) and an associated neurotoxin, domoic acid (Scholin et al., 2009). To our knowledge, that was the first instance of an ocean-deployable instrument being used to autonomously quantify species-specific sequences of nucleic acid as well as a corresponding cell metabolite.

The tandem use of the SHA and protein probe arrays aboard the ESP continues to this day, primarily for HAB research and monitoring. Improvements

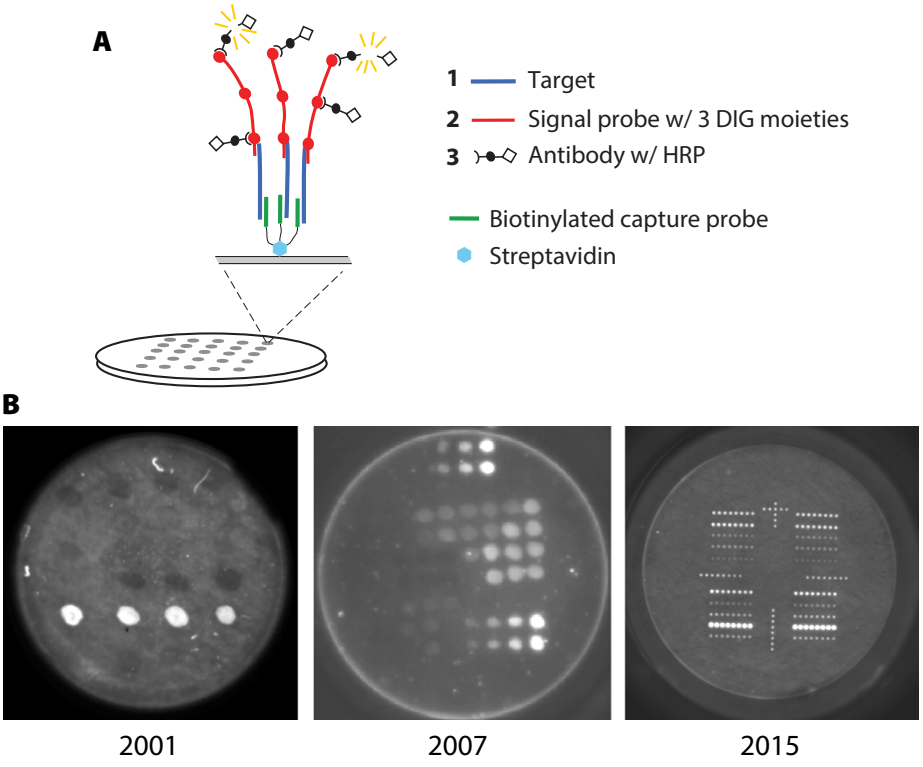

FIGURE 3. Evolution of ESP sandwich hybridization probe arrays. (A) Oligonucleotide DNA probes are attached to a nitrocellulose filter via biotin-streptavidin. When a sample homogenate flows perpendicularly through the membrane, the attached DNA probes capture their complementary rRNA sequences. Application of a digoxigenin (DIG) labeled signal probe then binds to sequences common among those retained on the array, directing an anti-DIG antibody/horseradish peroxidase (HRP) conjugate to the retained rRNA "sandwiches." When a chemiluminescent HRP substrate is applied, light is emitted in proportion to the abundance of retained signal probes, hence targeted rRNA sequences (Greenfield et al., 2006; Preston et al., 2009). (B) Early arrays were created by hand-spotting "probe ink" solutions onto filter membranes, and then later were improved by using automated devices such as Scienion's sciFlexarrayer S3 to improve the printing method.

in ESP deployment platforms, communications infrastructure, and data dissemination tools now make it possible to broadcast results of HAB species and toxin assays in near-real time via the Internet. Currently, that technology is routinely applied by teams at the Woods Hole Oceanographic Institution, NOAA's Cooperative Institute for Great Lakes Research and National Marine Fisheries Service, and MBARI as one component of larger observing systems that are in many ways conceptually consistent with what was envisioned decades ago (Figure 1; e.g., Ryan et al., 2011, 2017; Seegers et al., 2015; Bowers et al., 2016).

RETHINKING THE ECOGENOMIC SENSOR SOLUTION

As the ESP matured, it became clear that elements of the instrument's design were inherently limiting. For example, quantitative polymerase chain reaction (qPCR) had become a primary analytical tool for marine microbiologists (e.g., Church et al., 2005). That versatile technique made it possible to detect low-copy-number genes that were central to driving particular biogeochemical transformations (e.g., nitrogen fixation)—numerous genes of interest that the ESP's SHA array could not measure. The pressure rating of the ESP sample inlet was also limiting; the instrument was operable to a maximum water depth of 50 m, but many potential applications required going much deeper. How could the needs of expanding the analytical utility and operational range of the instrument be met without undertaking a complete redesign?

The push for additional functionality introduced the idea that ecogenomic sensors would ideally be comprised of a series of standalone components that could be assembled to create tailor-made (i.e., modular) systems for operating in different environments and conducting different types of analyses (Scholin et al., 2009). A "core instrument" was at the center of this notion, and "external sampling modules" could be added to ameliorate any sample intake limitations the core might have (e.g., operating pressure). Likewise, the addition of "analytical modules" could enable different types of molecular analytical assays that the core system could not support singularly. Notionally, by standardizing the sampling and analytical modules' fluid, power, and communications interfaces, it would be possible to realize a "plug-and-play" ecogenomic sensor system. Once again, this concept proved far easier to imagine than to operationalize.

Initially, the ESP was designed to function at pressures found from near the ocean surface to 50 m depth. Operations outside of that range needed additional sample handling, a requirement that gave rise to the development of sampling modules, which evolved for deep water as well as for shore-based operations (Figure 4). A deep water sampling module (DWSM)

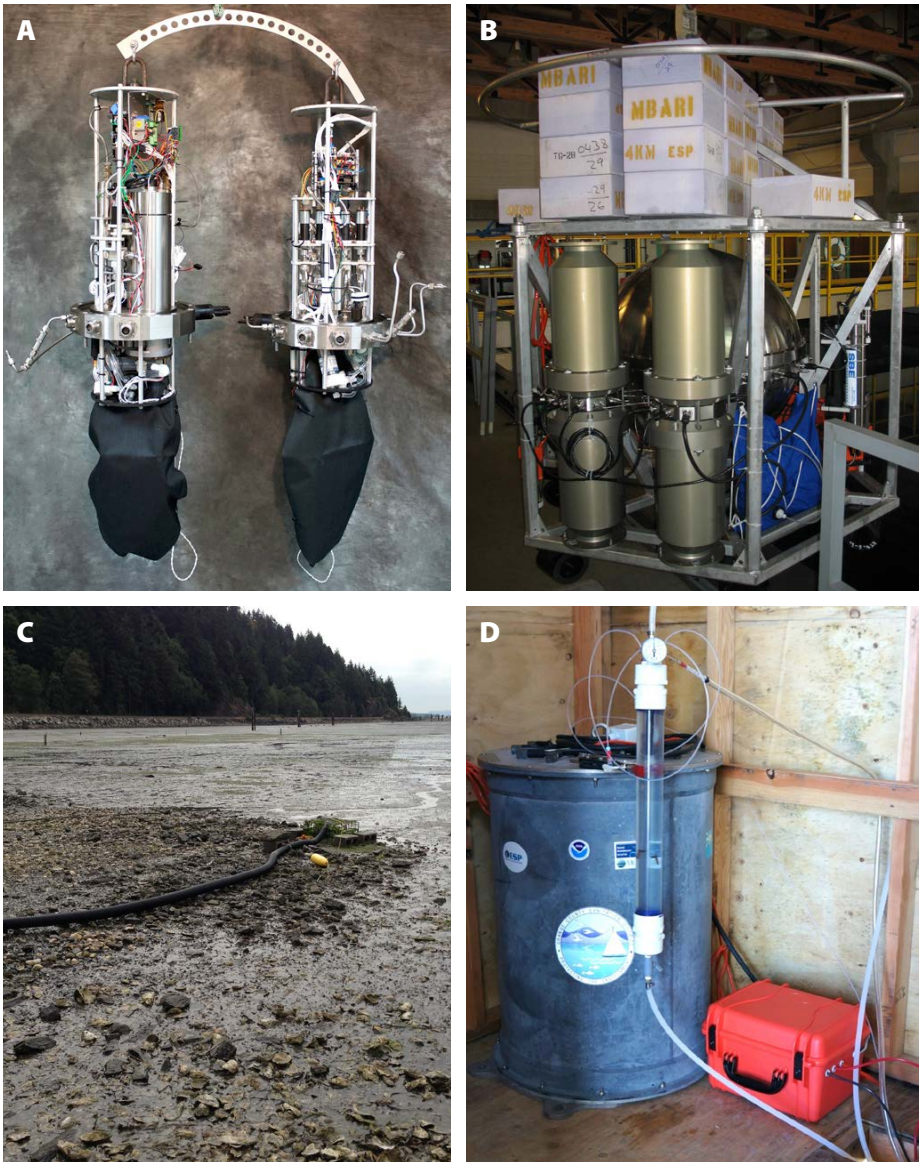

FIGURE 4. External sampling modules for the present-day ESP. (A,B) A deep water sampling module (DWSM) is used to collect water between 50 m and 4,000 m depth, and depressurize it to 1 atm before introduction into the ESP. The DWSM components (A) are located in their own set of cylindrical pressure housings (B) and allow the ESP as shown configured to operate in the deep sea (Ussler et al., 2013; Olins et al., 2017). (C,D) For above-surface applications, a tube is often run to the sample collection site, such as shown here at a shellfish farm at low tide (C). Water is pumped to an accumulator (D), which raises the water pressure slightly to aid in filtering through the ESP (Yamahara et al., 2015; Herfort et al., 2016).

was devised to acquire up to 10 L at depths to 4,000 m and then depressurize the sample by expanding its volume before passing it to the core ESP (Ussler et al., 2013; Olins et al., 2017). Other types of sampling modules were developed to bring water to the ESP when the instrument was near but not in the water (e.g., housed on a pier or other facility near the water's edge). Those modules created a slight positive pressure within a flow-through sample accumulator, which improves ESP sample collection when it is not submerged (e.g., Yamahara et al., 2015; Herfort et al., 2016). In both cases, the core ESP issues commands to the peripheral devices to initiate and coordinate sample acquisition.

The modularization concept was also applied to realize new analytical capabilities, in particular for deploying qPCR in parallel with SHA probe arrays. Although the core ESP integrates all steps, from sample collection through probe array analyses, it cannot accurately manipulate fluid volumes less than approximately 100 µL. For qPCR, at a minimum, precise and repeatable fluid handling at the scale of 1 µL is required. Thus, we created a separate fluid handling system dubbed the microfluidic block, or MFB (Figure 5); it can be operated independently or be attached to the core instrument via single electrical and fluidic connections. The MFB accepts sample homogenates generated by the core ESP, purifies DNA via a reusable solid phase extraction column, and then mixes necessary reagents to prepare material for a series of qPCR analyses (Preston et al., 2011). Because the sample homogenization method used for the SHA is compatible with the DNA purification technique, a qPCR-enabled ESP can direct a sample extract to either an SHA array or to qPCR analyses, or both in parallel. This capability was first demonstrated by deploying the ESP on coastal moorings (Preston et al., 2011; Robidart et al., 2012) and later on an open-ocean drifter (Robidart et al., 2014), revealing surprising variability in microbial community structure and function over short temporal and small spatial scales.

The creation of sampling and analytical modules opened new possibilities for exploring the use of the PCR-enabled ESP in deep-sea environments, as well as for onshore installations related to water quality monitoring. Ussler et al. (2013) documented the first use of the deep-sea ESP (D-ESP) at a methane seep in Santa Monica Basin. That work was followed by deployments at Axial Seamount (Olins et al., 2017) and on the MARS cabled observatory in Monterey Bay (recent work of author Preston and colleagues). Yamahara et al. (2015) expanded on the idea of using the SHA and qPCR capabilities in tandem to detect HAB species and microbial indicators of sewage effluents. Collectively, these studies show that the ESP can be applied in a range of settings, from basic research to resource management and public health protection initiatives. However, the instrument is relatively complex and expensive, and when fitted for PCR, it requires an expert-level operator. Clearly, to be applied widely outside of a research setting, it would have to be made much simpler to operate and less costly.

While the capability for conducting real-time analyses in situ has generated much excitement, those procedures are still limited by the power, size, and technology requirements needed for them to run unattended. Conversely, capturing and preserving material requires far less energy and technical complexity. Throughout the development of the ESP, we intended the sample preservation capability to be used to validate results of assays conducted in situ, in real time. Ottesen et al. (2013) expanded

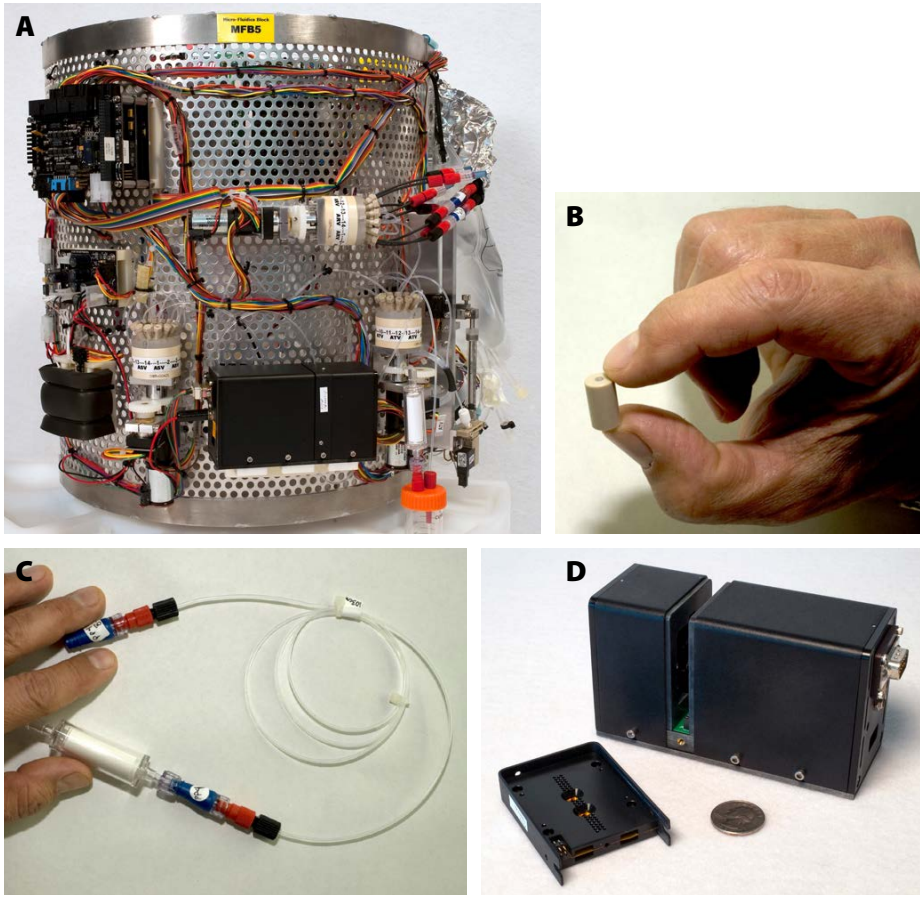

FIGURE 5. Designed to mount on the side of the present-day ESP (Figure 2A), the microfluidic block (A) can accurately manipulate fluid on the scale of microliters. Here, outfitted to perform qPCR (Preston et al., 2011), the microfluidic block contains its own solid-phase extraction column for purifying DNA (B), reagents stored at ambient temperatures in tubing coils (C), and PCR thermocycler (D) developed at Lawrence Livermore National Laboratory. US quarter dollar for scale.

on this idea by using the ESP specifically to collect and preserve samples for laboratory-based genomic and transcriptomic studies of marine microbial communities. In that case, there was no pressing requirement for real-time analyses, and the desired analytical methodology (high throughput DNA sequencing) was not available for use aboard an ocean-deployable, submersible instrument. Ottesen et al. (2013) found that sample processing required only the application of RNAlater® (Ambion Inc.); DNA and RNA were preserved with high fidelity even when stored in ESP pucks for up to one month at room temperature. This technique has since been applied extensively in coastal, open-ocean, and deep-sea ESP operations (e.g., Ussler et al., 2013; Varaljay et al., 2015; Herfort et al., 2016), expanding the utility of the instrument as a sample collection/preservation tool. For example, high-frequency sampling enabled by a free-drifting ESP was used to reveal diel patterns of coordinated gene expression among autotrophs and heterotrophs in coastal and oligotrophic ocean environments (Ottesen et al., 2013, 2014; Aylward et al., 2015). Interest in deploying the ESP to collect and preserve samples over long periods of time without servicing has since grown. Andrew Allen's group at the Scripps Institution of Oceanography recently found that high-quality RNA can be recovered from the ESP after six months of storage post-RNAlater treatment (after Ottesen et al., 2013), even when samples are held at or near room temperature (Andrew Allen, Scripps Institution of Oceanography, *pers. comm.*, December 2017).

TOWARD A NEW AND MOBILE ECOGENOMIC SENSOR

Our experience operating the ESP for different purposes and in a variety of coastal, open-ocean, deep-sea, and freshwater environments is helping to shape the design of a new version of the instrument. Field deployments of previous systems have been limited to moorings, a drifter, benthic landers, and shore-based as well as shipboard installations (Scholin, 2013). Such applications are not well suited to characterizing patchy communities of microorganisms because the number of ESPs that can be deployed at any given time is limited, and because the locations and depths of sample intakes are mostly fixed. In addition, the currently available ESP is a relatively large device, making it difficult to integrate with a variety of pre-existing autonomous and mobile platforms.

With these considerations in mind, we initiated an effort in 2009 to re-engineer the ESP with a goal of fielding the instrument on MBARI's 30 cm-diameter long-range AUV (LRAUV; Figure 6; Hobson et al., 2012). As in the past, the new instrument is designed for nucleic acid and antibody/receptor-based target molecule detection chemistries, as well as for sample preservation. Unlike its predecessors, however, the new ESP design concept is inherently more modular, with the functions of sample collection and handling separated from sensory systems needed for real-time molecular analytical analyses. In addition, the new instrument is no longer responsible for controlling external contextual sensors and transmitting data to shore; it is a standalone

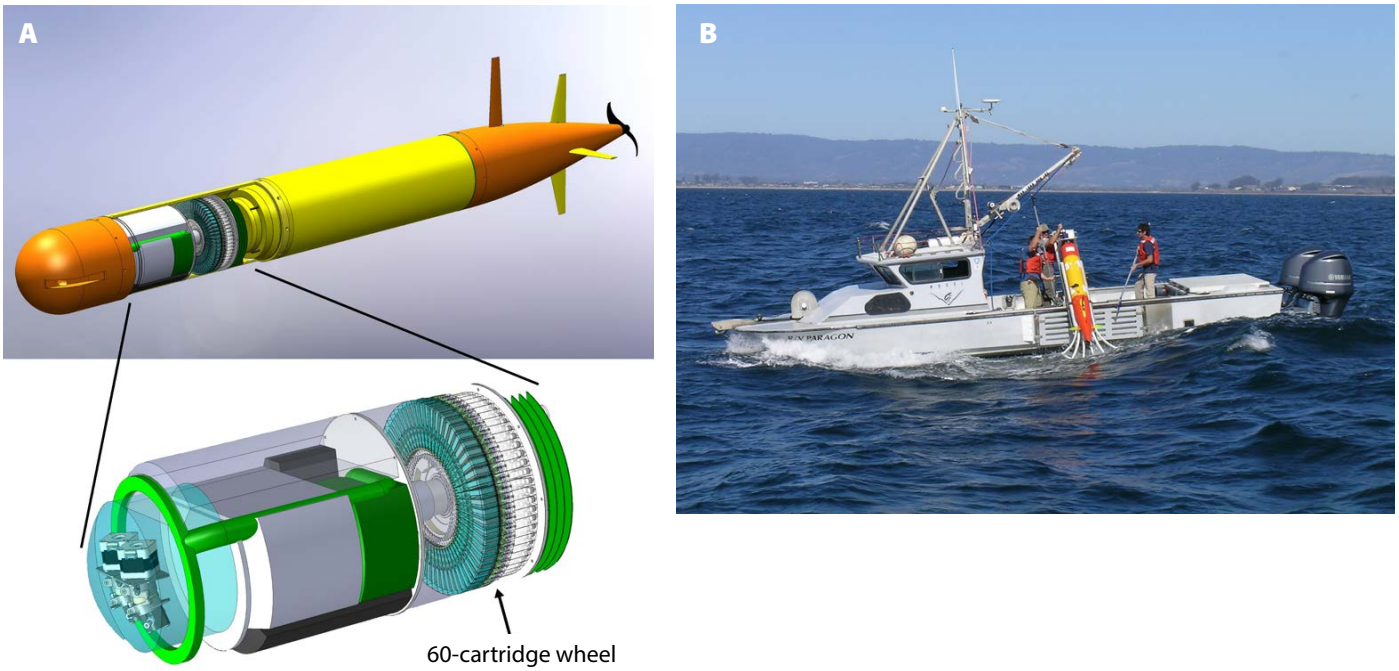

FIGURE 6. The next-generation ESP is designed to hold 60 sample collection cartridges and fit within a 30 cm diameter fuselage of (A) MBARI's long-range autonomous underwater vehicle (LRAUV). The cartridges are fitted onto a rotating valve assembly and align to a processing position where a single set of actuators (not shown) is used to divert sample through a cartridge and to carry out various steps of fluid handling. (B) The LRAUV can be deployed/recovered from small boats.

system that is prompted to initiate sample collection and analytical events by a separate, controlling platform.

Development of this instrument has presented a significant engineering challenge. Compared to the previous ESP, the new device had to be capable of meeting the same basic sample handling and processing needs while occupying roughly one-tenth the volume to operate at depths from the surface to 300 m, to be compatible with a greater variety of analytical methodologies, and to consume less power. Modularity allows for greater flexibility in configuring the instrument for specific uses, such as those requiring only sample preservation versus real-time analyses.

This new generation of ESP has many of the same functional requirements as the previous device, but the engineering solution for meeting those requirements is very different. In particular, we have adopted the concept of single-use "cartridges" that contain media for concentrating particles or dissolved substances, as well as the necessary reagents for processing that material for subsequent analysis (Figure 7). Compared to the existing instrument, housing the sample collection material and required reagents in a single cartridge reduces both the number of valves used to control complex fluid pathways and the need for common stores of reagents. With fewer common fluidic channels, the need to carry large volumes of cleaning and flush solutions is also minimized. The interface between the cartridges and the "core ESP sampler" has a fixed design. Cartridges can evolve to meet different sample handling and processing needs so long as they meet that interface specification, similar to how different pucks (Figure 2) are currently used.

Two types of ESP cartridges have been devised thus far: the archival cartridge (AC) for sample preservation, and the lyse-and-go cartridge (LGC) for sample homogenization (Figure 7). The AC is meant for sample preservation only; once sample materials are concentrated onto filter membranes, the preservative is applied. At this time, methods are in place for using the AC to preserve material for subsequent analyses that include qPCR, tag sequencing, and metatranscriptomics. In contrast, the LGC cartridge is designed for homogenizing collected material by application of lytic agents and heat; the resulting homogenate is passed from the cartridge to downstream analytical modules using a handoff mechanism that serves to make and break fluidic connections between individual cartridges. The LGC can produce homogenates suitable for use with surface plasmon resonance (SPR), qPCR, and lateral flow array analytical modules. These developments will be the subject of future communications.

> "Major strides in nucleic acid sequencing, mass spectrometry, and high-performance computing suggest that fully automated genomic, transcriptomic, and proteomic analyses for use onboard ocean-deployable instruments are realistic goals for the not-so-distant future."

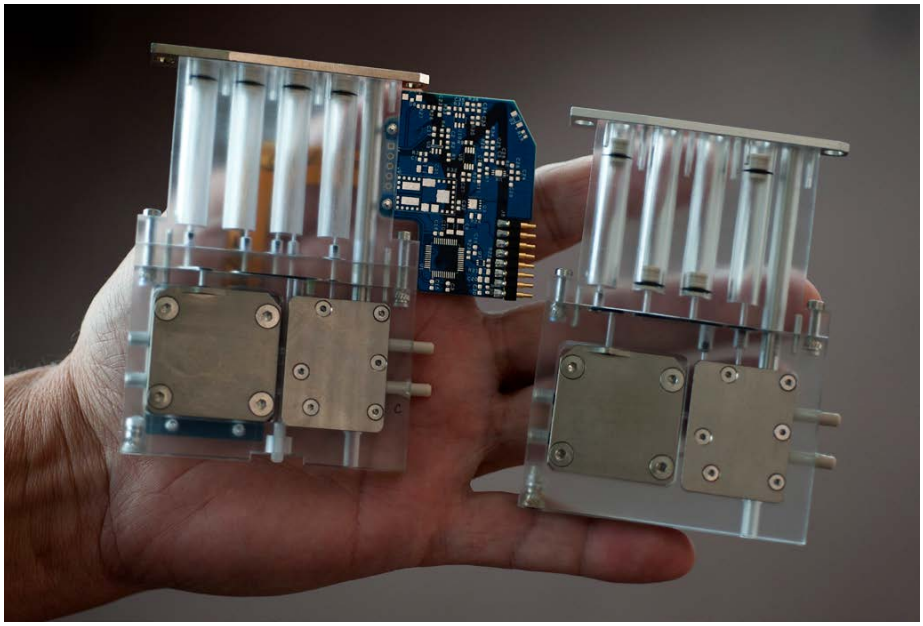

FIGURE 7. Next-generation ESP cartridges. (left) A "lyse-n-go cartridge" for collecting and homogenizing samples for real-time analyses, and (right) an "archival cartridge" for sample collection and preservation. Both cartridge types carry necessary reagents within one to three syringe barrels; the remaining barrels can act as collection chambers for waste or processed lysate. Note the increased complexity of the lyse-n-go cartridge, which requires electronics for heating to facilitate cell lysis, and a path off-cartridge for additional downstream processing within an analytical module.

As of late 2017, a 60-cartridge prototype of the new ESP has undergone laboratory tests and been integrated with the LRAUV for field trials. Efforts to deploy the first real-time analytical device have concentrated on a low power, miniaturized SPR module configured to detect

domoic acid. In addition, we have used the new ESP prototype to collect samples as the LRAUV undergoes different flight behaviors associated with different science use cases (e.g., Figure 8). For example, the vehicle can be directed to sample only when it finds a particular feature in the horizontal or vertical planes, while it's drifting at a depth, during level flight, or spiraling from one depth to another to collect an integrated sample over a depth profile.

A GLIMPSE OF THE FUTURE

For the past 25 years, MBARI has worked to develop an instrument that exemplifies the ecogenomic sensor idea, proving its viability, commercializing the first working example of the device, and providing support for its operation at various locations around the world. Over that time the ecogenomic sensor concept has matured, but in many ways elements of what was imagined over 20 years ago have indeed become reality. Despite that progress, this class of instrument is still very much in its developmental infancy when compared to other commercially available devices that are used routinely for environmental research and monitoring purposes (e.g., CTD, fluorometer, oxygen and pH sensors). For the ESP, several near-term opportunities for applying and further developing the technology are on the horizon: expanding the capacity to collect environmental DNA (eDNA, genetic material shed or excreted by animals); improving sample collection capabilities needed to resolve complex biological processes associated with episodic events; and enabling small, autonomous mobile platforms to carry out increasingly sophisticated types of molecular analyses in situ.

Comprehensive sequence analysis of eDNA is an intriguing approach for determining the diversity and relative abundance of animal communities absent direct observations of the animals themselves (Kelly et al., 2014; Port et al., 2016; Andruszkiewicz et al., 2017). Indeed, eDNA analyses are anticipated to become a cornerstone of future marine research, resource management, and conservation initiatives. Beyond using traditional manual and ship-based sampling methodologies, how will sample collection be scaled up to meet demanding areal and temporal eDNA survey needs? A remotely operated, programmable, and mobile sampling capability offered by the ESP-LRAUV offers one opportunity for meeting that need.

Similarly, it is still challenging to capture samples of microbes associated with short-lived (several day) stochastic events far from land that contribute significantly to nutrient cycling, total primary production, and carbon export. Near-monthly observations carried out via the Hawaii Ocean Time-series (HOT) program exemplify that notion, especially for processes operating on diel to weekly timescales (e.g., Guidi et al., 2012). If comprehensive observational programs like HOT are inherently undersampling, what can be done to improve our understanding of the metabolic balance in the sea? A fleet of AUVs offers one means of addressing that question by providing a platform for acquiring physical samples at high frequency in relation to dynamic chemical and physical features present in the ocean. With that objective in mind, a cohort of three ESP-equipped LRAUVs, dubbed MiVEGAS (Multiple Vehicle EcoGenomic Automated Samplers), are under construction now at MBARI and are slated for delivery to researchers at the University of Hawaii for operation beginning in 2018 (Figure 9A).

Increasing the use of ecogenomic sensor technology to obtain real-time molecular analytical measurements is another area where advances await, particularly in the context of resource management and public health protection. How can we capitalize on this idea? Such work benefits greatly from collaborations that drive the science, technology, and

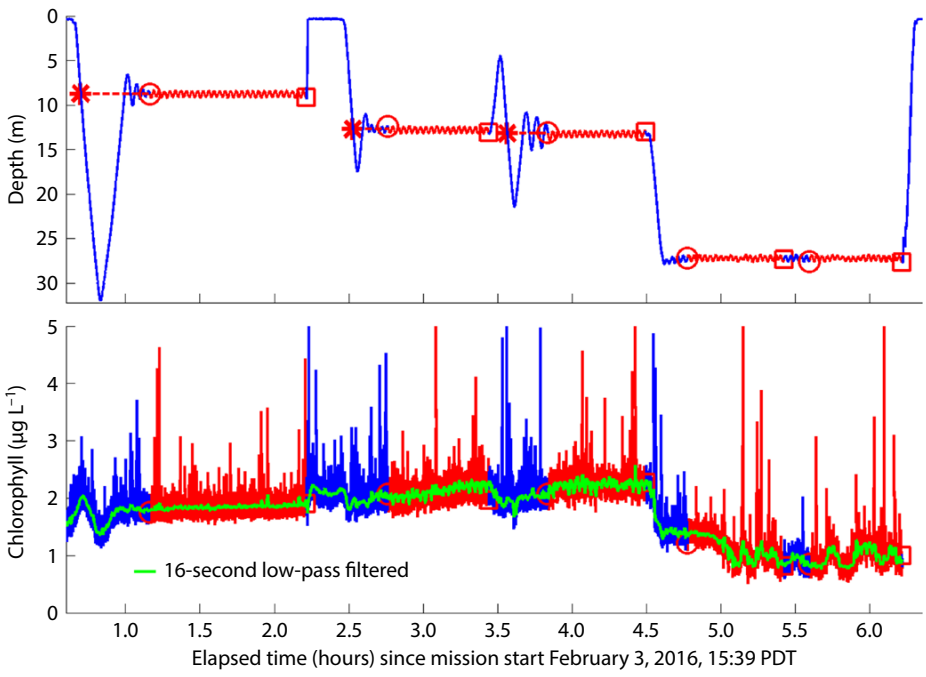

FIGURE 8. The *Makai* ESP/LRAUV demonstrated autonomous detection, sampling, and processing in a subsurface chlorophyll layer followed by surfacing to report its findings to shore. Vehicle depth (top) and measured chlorophyll concentration (bottom) were recorded during a five-cartridge collection experiment. The vehicle was instructed to find a chlorophyll maximum layer, drift within that feature, collect a sample (hrs. 1.1–2.25), lyse that sample and perform surface plasmon resonance (SPR), then report back to surface once complete. Then it was instructed to relocate and drift with the chlorophyll feature and collect two archival samples, reacquiring the peak signature prior to initiating the second sampling. Without resurfacing, it was then programmed to dive to a specific depth below the feature and collect two more archival samples (note a slight diminution in chlorophyll concentration during the final two samples) before finally surfacing for recovery.

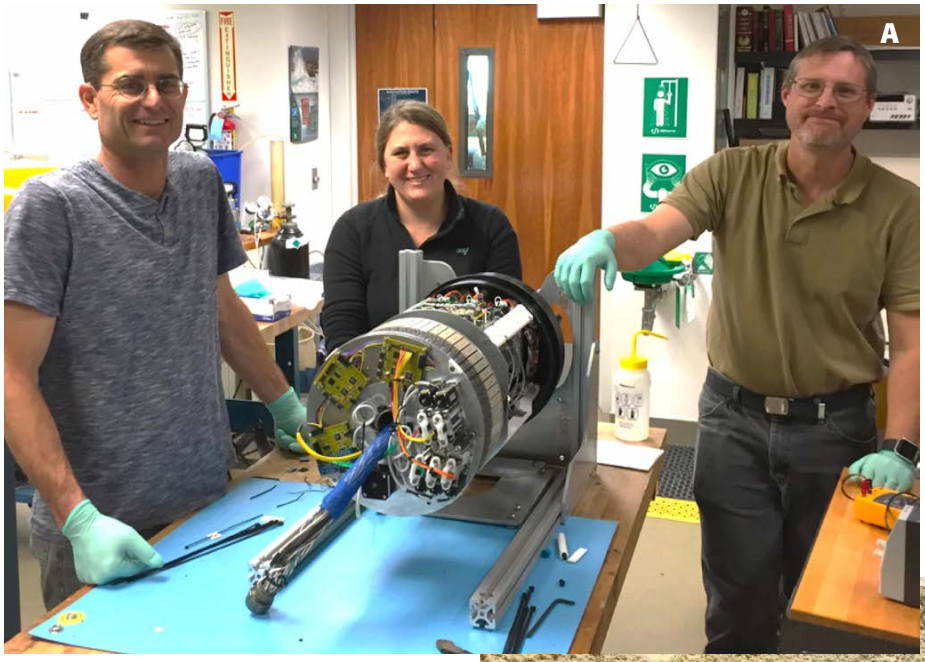

FIGURE 9. (A) MBARI electrical engineer Scott Jensen (left), research specialist Chris Preston (center) and mechanical engineer Doug Pargett prepare the next-generation ESP for integration with the LRAUV. *Image credit: Chris Scholin* (B) MBARI Senior Research Technician Roman Marin III uses a hand-portable version of the newest ESP to collect water samples from the Gardner River in Montana in a collaborative experiment sponsored by a USGS Innovation Center grant (see https://www.mbari.org/mbari-biologists-on-the-trail-of-brain-eating-amebae). *Image credit: Kevin Yamahara*

application fronts simultaneously. For example, using the LRAUV to find features that may harbor toxic algae below the ocean's surface as a means to trigger the ESP-SPR system to assess the presence and abundance of toxins and responsible species would open a new window to HAB research and mitigation. Likewise, the use of qPCR and probe array methods would provide a complementary way to survey coastal waters for indicators of sewage effluents, pathogens, metabolites, and other harmful constituents. As capabilities evolve for biomedical and point-of-use commercial applications, new sensing modalities will undoubtedly become available. Adopting such technologies for environmental research will require a capability for acquiring and processing relatively large samples "upstream" of analysis. The design of the ESP is evolving to meet that common need; such applications will demand that the sampling system be very compact and energy efficient, consistent with a device that is ultimately hand-portable (e.g., Figure 9B).

Analytical advances made in the cellular and molecular biology fields have long served as catalysts for breakthroughs in how we observe life in the sea. Consideration of that historical progression along with its future trajectory offers insights into how ecogenomic sensor technology may evolve in the future. Major strides in nucleic acid sequencing, mass spectrometry, and high-performance computing suggest that fully automated genomic, transcriptomic, and proteomic analyses for use onboard ocean-deployable instruments are realistic goals for the not-so-distant future. Ironically, none of the promises that ecogenomic sensors hold will come to fruition unless the age-old challenge of acquiring and processing representative samples is first met.

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