Development of a Mobile Ecogenomic Sensor

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Abstract— Modern ocean microbial research utilizes advanced molecular analytical techniques, such as polymerase chain reaction (PCR), DNA and protein probe arrays, and nucleic acid sequencing (etc.). Applying or at least initiating these techniques at the point and time of sample collection can enhance their effectiveness. To that end, in-situ sample processing and real-time molecular detection schemes have been implemented using deployable autonomous systems that can be operated in diverse ocean environments from shallow coastal waters to the deep sea. Such devices have been termed "ecogenomic sensors." The size of these instruments currently requires that they be moored in a fixed location or passively mobile, drifting at fixed depth and observing microbial communities in a moving frame of reference with ocean currents.

With the highly dynamic motion of open water and microbial life, the next frontier of ocean microbial research requires the improved capability of an actively mobile asset. A mobile ecogenomic sensor encompasses a fully maneuverable vehicle with weeks of persistence, environmental data analysis, detection of physical and biological features, autonomous sampling and in situ analysis, and near-real-time data reporting. This system is now being developed by integrating three components: a compact molecular analytical instrument (the 3rd generation Environmental Sample Processor), a long-range autonomous underwater vehicle, and software algorithms for AUV-based feature detection and sampling. A summary of the system and its initial application is presented.

Index Terms—Autonomous underwater vehicle (AUV), ecogenomic sensor, harmful algae bloom, in situ instrumentation, microbe, PCR, sample collection.

I. INTRODUCTION

Scientists routinely employ a suite of molecular analytical methods to investigate the diversity, activity and response of marine microorganisms in relation to natural and anthropogenic environmental perturbations [1,2]. Those methods include use of the Polymerase Chain Reaction (PCR), DNA probe arrays, DNA and RNA sequencing, and detection of specific metabolites and lipids – techniques that

are commonplace in most all modern environmental science and medical laboratories.

While the utility of molecular analytical methods is well established, a fundamental limitation of their application in a marine setting is the acquisition of the physical sample for analysis. Manual sample collection can be expensive, requiring ships that can locate and get to the area of interest, as well as remain there until enough samples have been acquired to adequately capture the process of interest. Thus, time on-site is limited and sporadic, and access to remote locations is particularly challenging due to ship scheduling and the expense associated with extended ship operations.

Additionally, traditional sampling schemes require people to handle the water and manipulate the material collected, which is then followed by transporting the collected samples to a laboratory for final processing. The handling and transport introduces a time lag of several hours or even days between sample collection and analysis. This not only makes acquiring the data in near-real time either impractical or impossible, but can also compromise sample integrity since microbes can undergo rapid change post-collection thus obscuring the actual state of the community when the sample was acquired. This time lag between sampling and data can make it difficult to analyze and respond to dynamic or stochastic events in the ocean.

II. ECOGENOMIC SENSORS

The difficulty with returning samples to the laboratory is the driving force behind the development of systems where collection, processing, and analysis of discrete samples occur immediately in the environment of interest (i.e., *in situ*).

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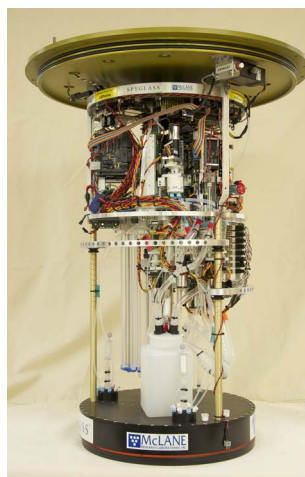Email: dpargett@mbari.org

Fig 1. Photo of the second generation (2G) ESP core instrument, without reagents and PCR module.
It is shown fitted with a surface mooring pressure housing top cap. The 2G ESP is 55cm diameter and 81cm tall. The instrument is protected under U.S. Patent No. 6187530 [3].

With such a system, ocean conditions can be monitored continuously, and when conditions warrant, sample collection and processing can be initiated absent a direct human presence. A class of device that embodies these capabilities, integrating genetic level analysis with larger scale environmental characterization, has been termed an ecogenomic sensor [4].

Fig. 2: Size comparison of two ecogenomic sensors.
Top: 2nd Generation ESP, loaded with reagent bags and media filters, is being lowered into a 215L pressure housing for deployment on a mooring.
Bottom: Long Range AUV *Makai* with 3G-ESP assembled for deployment. The marked section of the AUV contains the G3-ESP instrument in a 40L space.

A type of ecogenomic sensor developed at the Monterey Bay Aquarium Research Institute (MBARI) is the Environmental Sample Processor (ESP). The ESP is a submersible robotic instrument that performs real-time, autonomous sample acquisition and processing for application of DNA and protein probe arrays, as well as quantitative PCR (qPCR), to detect microorganisms as well

as phycotoxins [4,5,6,7]. This technology has been in development for over 15 years, having evolved from 1st to 2nd generations of the instrument. The latter, or "2G ESP" (Fig 1, 2 top) is commercially available from McLane Research Laboratories (Falmouth, MA).

The 2G ESP is used by a variety of academic, government, and private institutions that utilize the device in marine and freshwater settings for basic research and resource management purposes. The most common 2G ESP ocean deployment architecture is a taut mooring, with the instrument sitting 5-25m below the surface, tethered to a small surface buoy that provides satellite or cellular telemetry. A passive drifting platform using a 2G ESP instrument has been utilized to more effectively observe variations of microbial communities in their natural moving frame of reference [8,9,10].

After 7+ years of successful deployments and operation, one identified shortcoming of the 2G ESP is its size and limited mobility configuration. Because the 2G ESP is anchored to the seafloor, or set at a fixed depth, it is relatively stationary and as such, the organisms and compounds detected are only those immediately adjacent to the instrument. Microbial populations in the ocean commonly form layers and patches. Any microbial populations just out of sampling range, above or below the instrument will not be detected or may be dramatically under-reported (e.g. Fig. 3).
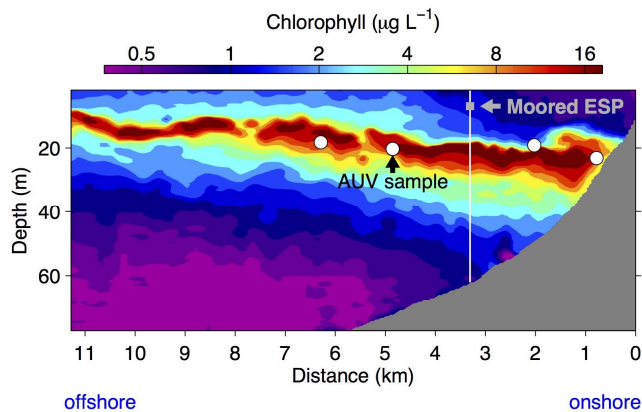

Fig. 3: Toward targeted ecogenomic sensing. This interpolated vertical section from 28 May 2015 shows chlorophyll concentrations mapped by the *Dorado* AUV in southern Monterey Bay during a highly toxic phytoplankton bloom. When winds subsided, toxic cells submerged and formed a densely concentrated subsurface layer, below the moored ESP. Autonomous detection and sampling of the layer by the *Dorado* AUV [11] returned whole water samples from the layer. To enable better sampling of such phenomena, 3G ESP integrates the autonomous real-time molecular analysis with the persistence, mobility and intelligent sampling capabilities of a long-range AUV.

Additionally, since the ocean is a dynamic environment with microbiological processes that evolve in both space and time, the samples and data collected by a moored ESP are actually indicative of what occurred "upstream". Consequentially, consecutive samples collected from the same geographic spot some period of time apart may be from separate water masses, and are not indicative of how a

particular microbial population has changed over time in response to environmental perturbations. Tracking coherent communities over an extended period, as well actively searching for and sampling different communities as they are encountered, have thus emerged as key science requirements not easily met using the current 2G ESP system.

The questions concerning space/time relationships of microbial communities have led to requirements for sampling across "boundaries" indicated by a change in environmental conditions (temperature, salinity, nutrient, etc.) to better understand why microbial populations may be present under certain conditions but not others. These needs have a common solution, controllably moving the sensor through the ocean to find and then sample around the area of interest. These emerging capabilities have led to the conceptualization of a new class of mobile ecogenomic sensor that could execute "seek, find, follow, and sample" missions. This mobile sampling system must be able to remain at sea for extended periods, be directed from shore with minimal human intervention, perform various sample collection routines, apply molecular identification techniques autonomously, and range freely in the upper water column to a depth of at least 300m.

III. LONG RANGE AUV

An autonomous underwater vehicle provides an ideal mobile ocean platform, and for this development the Tethys class Long Range AUV (LRAUV) provides key capabilities: large energy storage and low consumption allows for deployments lasting a few weeks and ranging up to 1800 km; a variable buoyancy system allows the vehicle to ballast to neutral buoyancy, holding at a fixed depth and drifting with the water mass [12]. A suite of sensors that can measure the temperature, salinity, depth, oxygen, nutrients, chlorophyll, and other environmental data, allow the vehicle to record the ocean conditions and navigate track and dive based on these measurements, enabling the "seek, find and follow" mission tasks.

Autonomously maneuvering the vehicle based on local environmental conditions is also critical to the "sample" mission task, which may commonly require sampling at multiple locations to provide contrasting data. Samples might be collected from the center of, above and below a dense chlorophyll layer, or on opposite sides of a thermal boundary, such as may indicate a cold water upwelling [13].

The 3m long, 160 kg size of the Tethys class LRAUV (3G ESP configuration, Fig. 2 bottom) simplifies deployment logistics as it can be deployed from small boats. However, this size also posed a challenge for integrating a complex genomic data instrument.

IV. THIRD GENERATION ESP

The large size of the 2nd generation ESP instrument is prohibitive to almost any AUV platform, therefore we designed a "3rd generation" ESP (3G ESP, Fig. 4) to fit in a 30 cm diameter, Tethys-class LRAUV.

The patent pending 3G-ESP meets the same set of fundamental requirements as its predecessor, but it is much more compact and modular, with a deeper operating depth (300m) capability. The new design is built around the notion of a "front-end" sampling system that utilizes independent cartridges to collect and concentrate microbes, particulates, and/or dissolved substances on varied media. The sample collection media along with the fluidic paths, devices and processing reagents for one sample are contained in a cartridge. Depending upon the type of cartridge, the sample can be preserved for later analysis in the laboratory, or processed immediately by the instrument and prepared for "back end" analyses utilizing add-on analytical devices that connect to the cartridges via a standardized interface.

The instrument is designed to function as a standalone sensor that can be integrated with multiple platforms, the LRAUV being one example. It remains powered off until an external controller determines that it is an appropriate time and/or location for collecting and processing the sample. Once completed, the 3G ESP informs the main controller, passing along analytical results if desired, thus "freeing" the host platform to resume its overarching mission.

Up to 60 cartridges can be installed on a circular wheel, with seawater ports connecting to a center ring of valves that provide each cartridge access to the seawater supply loop (Fig. 4). This cartridge wheel is motor driven and can be rotated so that any one of the cartridges can be aligned and locked in a position where a set of linear actuators may operate the fluidic devices of the cartridge. This location is called the processing position.
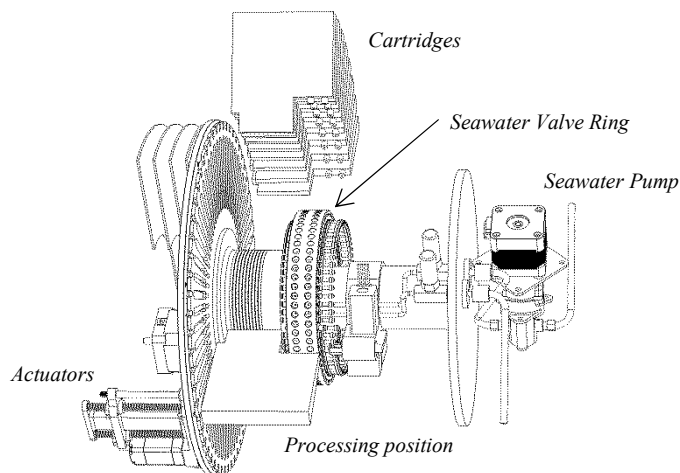

Fig. 4: Exploded drawing of the 3G-ESP instrument, showing cartridges and major components.

Between sample events, the seawater loop through the instrument is isolated from ocean pressure by two motor actuated quarter-turn valves. Sampling initiates when the valves are opened. The seawater loop through the instrument is pressurized by the ambient ocean as it is fed by a ceramic rotating piston pump, located outside the instrument housing in an oil filled pressure compensated chamber. A pressure

transducer measures the differential pressure between the pump output and the ambient ocean. This is used to control the pumping speed so that regardless of operating depth, the sample collection is performed at a constant pressure across the collection media. The differential sampling pressure can be controlled depending on type of collection media and the fragility of the organisms being collected.

The ring valves that feed the cartridges are a flow switching type arranged in series. When a ring valve is closed, the seawater flow is not blocked but bypasses that cartridge and continues around the ring to the next valve, and so on. Eventually, the seawater flowing in the loop is returned to the ocean. With all the ring valves closed, the seawater can be pumped through the loop, bypassing all the cartridges, allowing for flushing of the loop. When a ring valve is opened, the seawater flow is switched to the supply port of that cartridge, placing the cartridge's flow paths and collection media in the seawater loop. The cartridge return port brings the flow back to the valve ring where it continues around the ring, and is returned to the ocean. In this arrangement, the cartridges have numerical positions around the wheel, but equal access to the seawater loop so they can be used in any order.

Since the cartridges have independent reagents and fluidics, different types of cartridges with differing reagents may be utilized collectively for a deployment. The only requirement is that the cartridge interfaces to the instrument are common: seawater supply and return ports, actuator location, force and stroke, cartridge supply power and data, and product handoff port location.
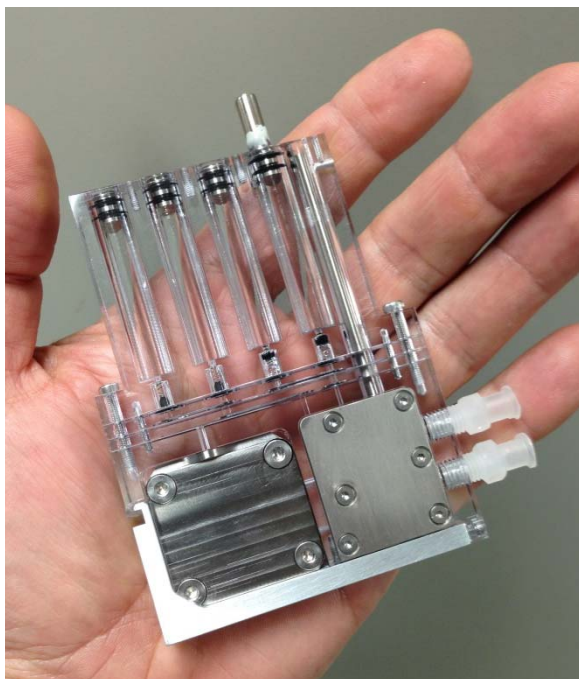

Fig. 6: Image of a prototype 3G-ESP sampling cartridge, containing the collection chambers, valves, and processing reagent reservoirs.

V.   Sample Collection and Processing

To perform sample collection, the ESP instrument initially pumps seawater through the ring of valves that supply the cartridges, flushing the loop of old seawater and cleaning fluid. The ring valve to the selected cartridge is opened, and a prescribed volume of seawater is pumped at constant pressure through the flow path, which includes the cartridge's collection media where particulates and/or dissolved substances are concentrated. The filtrate is returned to the ocean and the material retained on the collection media is the "sample" to be processed. After sample collection, the pump is stopped, the seawater loop is isolated from ocean pressure, and the ring valve is closed. The cartridge can now be processed.

Processing of the sample is performed by the instrument actuating the cartridge in a manner that either preserves and stores the collected material for later analysis, or liberates components for immediate analysis via one or more connected analytical modules. The cartridge wheel is rotated so the cartridge is aligned and locked in the processing position where the actuators can move valves and plungers to deliver reagents. Once the cartridge processing is finished, all the actuators are withdrawn from the cartridge and the cartridge wheel is unlocked.

A.   Sample Preservation

Since the platform can be deployed for many days, sample preservation provides a method of locking the sample's microbial population at that state, stabilizing it for later detailed analysis in a laboratory.

For processing of a cartridge for sample preservation, post sampling, a cartridge actuator switches the cartridge valve from sample collection to processing, allowing the reagents to be delivered to the collection media. Another cartridge actuator delivers a preservative reagent, such as *RNAlater,* to the sample, displacing the residual seawater through the collection media, and into the waste storage chambers on the cartridge. After a prescribed period of time the actuator cycles the preservation syringe, which pumps air into the collection media, and displaces the remaining preservative into the waste chamber. At this point, the sample is preserved on the collection media and actuators are withdrawn from the cartridge, and the wheel is unlocked.

Preservation in this manner stabilizes the DNA, RNA, and other microbial products so that the sample can be analyzed days or months following recovery of the instrument and removal of the cartridges. [14,15]

B.   Homogenate Processing

Cartridges that process samples for in-situ analysis have additional fluidic paths and a cartridge product port, which gets connected to an analytical module handoff system when the cartridge is locked in the processing position. A chemical process opens the walls of cells in the sample, releasing the cellular components (e.g. RNA, DNA, and proteins) into a stable fluid, creating a homogenate. When this homogenate is delivered to an appropriate analytical module, the target

molecules corresponding to specific genes, species or compounds will be detected if they are present.

For typical homogenate processing, the same sample collection is performed and the cartridge locked in the processing position, coupled to the handoff system. During processing, the instrument actuators switch the cartridge valve to processing and drive the cartridge pistons to evacuate the residual seawater from the collection media through the handoff to the instrument's waste chamber. Then an actuator delivers a biological or chemical lysis buffer reagent to the collection media. The process typically requires heating, so a local controller on the cartridge regulates the sample temperature accordingly via an embedded heater. After a prescribed heating time, the processing is complete and the homogenate can be delivered off the cartridge to an analytical module.

After the cartridge has completed processing and delivering the products; the actuators are withdrawn from the cartridge, the product port is decoupled, and the wheel is unlocked. The instrument is freed to process or preserve another cartridge. However the analytical module may continue to run, taking whatever additional time is necessary to complete the analysis of the delivered homogenate, communicating the resulting data to the ESP instrument.

VI. ANALYTICAL MODULES

A number of analytical modules are being developed that can be added to the ESP instrument to provide a variety of detection modalities in-situ; many of the specifics of these developments are proprietary at this time. The two most mature developments are analytical modules based on surface plasmon resonance (SPR) and PCR. SPR is a developed optical method using commercial devices wherein a range of targeted molecules can be quantified directly from a sample homogenate. This technique has been proven to provide a rapid, robust detection of a wide variety of microorganisms and compounds [16]. PCR allows for specific target detection using sequence-based DNA amplification and is widely applied in the environmental sciences [6, 17].

The data produced by in-situ detection can be relayed to shore, along with the environmental and sample context data, allowing science mission decisions, or even resource management or public health agency actions such as closing of beaches or facilities. However there is also the potential of utilizing analytical results onboard the platform to autonomously deliberate given an overall mission objective and thereby realize decisions that change vehicle operations and sampling parameters accordingly.

VII. FIELD TESTING

An engineering field test was performed with an early prototype of a 3G-ESP instrument, loaded with five preservation cartridges and mounted in the LRAUV *Makai*. It was deployed in Monterey Bay in July 2015 for five days with the goal of demonstrating a suite of fundamental operational capabilities required of a mobile ecogenomic sensor.

All five of the cartridges were utilized. Fig. 7 shows one autonomous mission to find a distinct chlorophyll layer, seek a sampling location (chlorophyll maximum in this case) and then follow that depth while sampling. That mission collected two additional samples, equal distances above and below the first (Fig. 7). The mission called for each sample to be collected from 1L of seawater, but taking no longer than 1 hour to collect and process. Due to heavy microbial load and fine porosity collection media, each sample collection required about 50 minutes to sample between 760 and 815 mL of seawater. At the time of this writing, the samples had not yet been analyzed in the lab to determine the specific microbial counts, or genomic information.
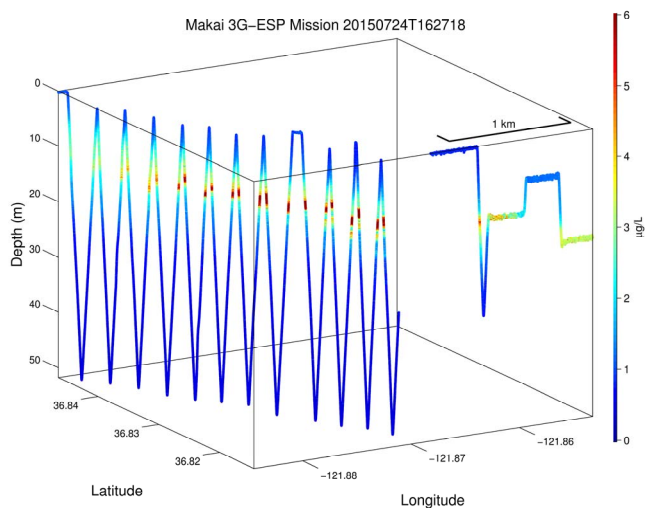

Fig. 7: 3D view of a transect by the LRAUV *Makai* during the test deployment. The chlorophyll concentration is shown along with depth and geographic location. On the right side, the sample collection mission shows the vehicle diving to locate the peak chlorophyll and then performing a triple depth sampling set, centered on that peak. The sample collection times are visible as the constant depth tracks.

VIII. CONCLUSION

The state of ocean microbial research and monitoring is limited by the expense and operating limits of manual sample collection using ships. Autonomous systems for sample collection, in situ processing, and preservation have greatly advanced this field. Deployment of these systems on moorings and drifters have proven effective for many aspects of microbial research, yet neither can fully meet the needs of sampling dynamic marine ecosystems with respect to both space and time.

New technology that can bridge these barriers is being developed by combining a new smaller ecogenomic sensor that provides sample collection and processing capabilities, a long-range AUV that provides persistence, and intelligent algorithms that provide targeted observation and sampling capability. A prototype instrument demonstrated this integrated functionality and was able to collect and preserve microbial samples from locations and times that were previously unobtainable.

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