Deep Water Instrument for Microbial Identification, Quantification, and Archiving

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***Abstract*—A deep ocean robotic platform capable of *in situ* microbial identification and quantification at depths to 4000 m has been developed. The platform is a free benthic lander, containing a low pressure microbial instrument and deep ocean sampling systems that can monitor and collect raw seawater from multiple sources, then decompress the raw seawater for processing by the detection instrument. This has enabled the autonomous collection, processing, and archiving of microbes from multiple sites. This allows for direct, time correlated comparison of different microbial populations along with the seawater physical and chemical composition.**

***Index Terms*— autonomous sampling, deep ocean, ecogenomic sensor, *in situ* instrumentation**

I. Introduction

Here, we report on the deep ocean application of a robotic system known as the Environmental Sample Processor (ESP) that conducts molecular biological analyses *in situ* as a means of identifying and quantifying a variety of microscopic organisms at oceanic depths down to 4000 m. The ESP concentrates the microbes found in seawater through filtration and detects target species using low-density DNA probe arrays, protein arrays [1-3], and real-time quantitative Polymerase Chain Reaction (qPCR) [4, 5]. Microbial samples, acquired *in situ,* can also be immediately preserved for further analysis after instrument recovery [6]. The deployment platform also carries external chemical and physical sensors to provide contextual measurements of the specific seawater being sampled, and of the surrounding environment.

The ESP performs all collection and analytical functions within a one-atmosphere pressure housing using fluidic equipment rated for only 500 kPa, therefore, several technical developments were undertaken to acquire deep water samples in the high pressure environment. This 4000 m depth-hardened version of the ESP is referred to as the Deep-ESP.

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The Deep-ESP platform is a benthic lander, designed to be easy to deploy, recover, and manipulate while on the seafloor. The Deep-ESP sample intake system can be deployed to specific locations nearby on the seafloor, safely collecting heated water from diffuse hydrothermal vents. The Deep-ESP power requirement is non-trivial, and a suite of electrical systems were implemented to reliably power the instrument, as well as reduce the consumption of shared expedition resources.

To prepare the Deep-ESP for deep water expeditions, we implemented a number of novel engineering solutions with special considerations. Here, we also report a set of preliminary data collected during the deployment of the Deep-ESP into the caldera of an active underwater volcano off the Oregon coast from July 15-20, 2011.

II. Pressure Housing

The core detection instrument in the Deep-ESP is a robotic fluidic lab that handles raw seawater and chemical reagents. It required years of engineering effort to develop and refine, however, the instrument is significantly larger than common deepwater pressure housings such as 43 cm glass spheres. Rather than attempting to miniaturize the core robotics, it was more effective for cost, risk, and schedule, to build a large pressure housing for the instrument. This spherical housing would be over 1m in diameter, machined from near-net-shape cast titanium, and hyperbaric tested to 41.4 MPa for ROV service to 4000m. It is the main pressure housing for the Deep-ESP system, but is also the major buoyancy element in the deployment platform, displacing 660 kg of seawater.

There are temperature constraints for the core ESP instrument necessary to maintain reagent viability. The instrument has to remain between 10˚C and 30˚C from the initial reagent loading, through shipping, during the deployment and even during the trip back to the lab for post deployment quality control runs. To maintain temperature while the instrument is deployed in the 2-4˚C deep ocean, it was more effective to setup a process for heating the sphere rather than try to develop and validate cold temperature reagents and processes. Therefore, the sphere has heaters, thermostats, and an insulation blanket just inside the upper hemisphere. When deployed, it requires an average of 50 W to maintain an air temperature inside the sphere about 10˚C above the ambient ocean. Note that this is the temperature of the air and instrument inside the sphere, not the sphere itself. Due to the very high heat transfer rates of liquid convection and metal conduction, the sphere is effectively at ambient temperature. Additional insulation could be added, but there is an upper temperature limit. All the power conversion and battery charging equipment is located in the sphere and provides waste heat to this space. While this has the benefit of utilizing the waste heat when deployed, it also presents an over-temperature risk when the sphere is sealed but not submerged in water.

III. *IN SITU* PRESSURE REDUCTION

To collect the particles for microbial detection, the core ESP instrument requires liters of raw seawater to flow through its filtration stage. However, the fluid handling components inside the instrument are rated only for 500 kPa service and cannot contain ocean pressures when deployed deeper than 50 m.

For the same reason that we did not try to miniaturize the ESP, it was intractable to modify the robotics and fluid components to directly sample water at 4000 m depth. Instead, we developed a pressure-modifying interface called the Deep Water Sampling Module (DWSM) which allows the ESP instrument to sample seawater from a low pressure envelope while it is deployed in the deep ocean.

The DWSM functions as a decompression gateway by drawing in seawater (up to 10 L) at ambient pressure, decompressing the sample to the instrument's pressure, and feeding that sample through the filtration stage. The filtrate water coming back from the instrument is collected in a downstream reservoir, recompressed, and exhausted back to the ocean.

The DWSM assembly is two tower structures outside of the main pressure sphere, and has flexible water reservoir bags housed in rigid oil chambers (Fig. 1). The system has oil pumps, valve manifolds, and an oil reservoir at ambient pressure. The oil pumps create differential pressure between the chambers and the ambient ocean for filling and empting the seawater reservoir bags. When the DWSM is closed to the ocean, a high pressure pump can pump oil from the chambers out to the ambient oil reservoir. This reduces the pressure inside the chambers and creates the decompressed state of the seawater sample. The pumps only contact oil (silicone oil in this case) and do not suffer corrosion from seawater, nor are microbes in the seawater potentially destroyed if they were to pass through a pumping mechanism. Recompression of the system is accomplished by allowing the ambient pressure oil to flow back into the chambers via a restriction valve.

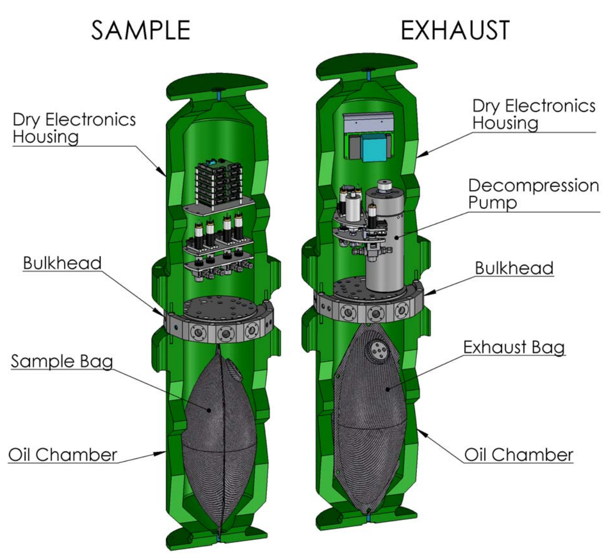

Fig. 1. Cutaway view of the DWSM tower architecture, showing pressure chambers, bulkhead plates, and major components.

IV. BENTHIC LANDER

When fully assembled and prepared for deployment, the Deep-ESP system is a free-fall benthic lander, about 2.5m tall and 2m in diameter (Fig. 2). This assembly contains the ESP sphere, DWSM, batteries, buoyancy foam, and a drop weight release system. There is a seawater intake and wand system that can draw water from multiple source points around the lander, this system includes physical and chemical sensors that provide additional context about the seawater being collected. Typical sensors are an In Situ Ultraviolet Spectrophotometer (ISUS), an In Situ Mass Spectrometer (ISMS), and a CTD with fluorometer and oxygen sensors.

The lander has beacons for tracking and an acoustic modem

Fig. 2. Deep-ESP lander being tested in seawater tank prior to deployment. The titanium sphere houses the ESP instrument (center); the green towers are the DWSM (left); the dark bag in front of the sphere is the ambient oil reservoir (center bottom); the orange boxes are batteries (right); and syntactic foam blocks trim buoyancy (top).

for ship-to-instrument communication while deployed. The modem can also trigger a galvanic corrosion wire that releases the lander's drop weight. VHF and GPS tracking system are also used to locate the instrument when it returns to the surface. There is also a platform interface cable for connecting the Deep-ESP to a submarine cabled observatory, ROV or an external battery pack.

The total mass of the Deep-ESP lander varies with the mission equipment and number of batteries on board. For the 2011 Axial seamount mission, the lander massed 1930 kg. The lander's weight in seawater is trimmed by adding or removing syntactic foam blocks so that it is 350-530 N (equivalent to 36-54 kg) heavy during descent. After releasing the 119 kg steel drop weight (100 kg equivalent mass in seawater), the lander becomes 450-630 N (equivalent to 46-64 kg) buoyant and freely ascends to the surface. The lander has been designed to have the center of buoyancy much higher that it's center of mass during both descent and ascent. This gives the lander a very strong righting moment, and it will return to upright if any external forces act to tip it. During descent and ascent, the Deep-ESP lander travels through the water column at about 30m/min.

V. Sample Intake and Wand System

A significant capability of the Deep-ESP system is that it can sample different water patches within a local area around the lander, enabling the comparison of microbial populations at various seeps, vents, and ambient water. This intake system is composed of a tree of centrifugal pumps and check valves, with each pump pulling water from a separate source location. Each pump is able to supply separate source water to a common supply header that feeds the ESP instrument (via the DWSM) and the context sensors (Fig. 3). These pumps are selected by the Deep-ESP, and such switching of sources does not require ROV servicing to reposition the instrument or intake wand.

Only the water supplied by the one pump being powered is fed to the supply header, and the check valves prevent back flow of water to the other sources. After passing through the last context sensor, the water is exhausted to the ocean about 0.5m above the sea floor, on the opposite side of the lander from the typical ambient seawater source, which is ~2m above the seafloor.

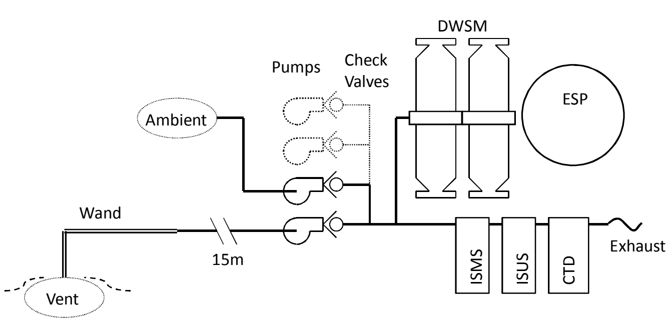

Fig. 3. Diagram of the Deep-ESP multiple source intake and wand system. For Axial seamount, it was configured for two sources, vent water (via a wand) and ambient water near the top of the lander. The system can be expanded to accommodate more source pumps.

The microbes at diffuse thermal vents are an intended research target for the Deep-ESP; however, the temperature limits of the instrument require that the lander be staged in cold ambient water, preferable some short distance from the vents. Therefore, a wand assembly has been developed which attaches to a pump and allows the intake of seawater from a source up to 15m from instrument. The wand head can be placed into cracks and vents to sample that specific water, and a screened intake excludes particles larger than 0.5mm, which could clog the ESP instrument. The wand head is a 1.5m titanium tube which acts as a heat exchanger, cooling the vent water prior to the flexible tubing and lander.

The wand head also contains a remote temperature sensor, to record the water temperature at the source collection point. This temperature is monitored in real-time via an auxiliary input channel on the CTD instrument.

VI. Power and Energy Storage

The Deep-ESP is not a low power system, and depending upon the mission plan and context sensors it averages about 170 W, however, it has been designed to make the best use of that power. Table 1 shows the major power consumers, averaged over 24 hours. The system is designed to typically be powered from a continuous supply, such as a submarine cable, however, for very short periods that supply may become insufficient. When the high pressure pump decompresses the DWSM, the power draw can be over 600 W for a few minutes, so the lander has onboard batteries to buffer the incoming power. The lander can be fitted with up to 8 kW-hr of pressure compensated lead acid batteries, which also enable the Deep-ESP to operate for about 48 hours at locations where there is no submarine power.

TABLE I
AVERAGE POWER CONSUMPTION BY SYSTEM
(GREATER THAN 10 W)

ESP Instrument and DWSM	20 W
Sphere Heater	50 W
ISMS (In Situ Mass Spectrometer)	100 W

Because of the instrument's power demands and the lack of submarine cable power at Axial seamount for the 2011 deployment, we used *in-situ* charging processes to augment the onboard battery power. To get high bandwidth data from the Deep-ESP, the typical mission profile calls for using an ROV to connect to the Deep-ESP almost daily for Ethernet and power, similar to what a cabled observatory would provide. During this "plug-in time" battery chargers in the sphere can use the ROV supplied power to back charge the lead acid batteries on the lander. For the Axial deployment, the ROV could provide a maximum of 500W to the lander, but since the platform would continue to consume 170W, only 330W could go into charging, subject to efficiency losses. In practice, devoting the entire ROV dive to just to charging would not keep up with the 24 hour battery consumption; therefore, an additional power system was developed.

VII. Battery Sleds

Battery sleds extend the D-ESP operating time and allow for more flexible use of the ROV, as it could be used for other activities during the Deep-ESP deployment rather than just charging batteries. Each sled stores about 4 kW-hr in pressure compensated lead acid batteries and fits into one half of the ROV tool drawer, an extendable storage drawer on the front of the ROV. Two battery sleds are used; a charged one is carried down by the ROV to the Deep-ESP where it will replace the discharged battery sled (Fig. 4). After the ROV has plugged into the Deep-ESP and downloaded the previous day's data or updated the mission, the new battery sled gets plugged into the Deep-ESP and the discharged sled is loaded into the tool drawer. When the ROV is recovered each day, the battery sled stays in the tool drawer and is recharged overnight.

The Deep-ESP's circuitry prioritizes power from the battery sled over the lander's onboard batteries. In theory, a fully charged battery sled contains nearly 24 hours of energy for the Deep-ESP and a daily battery swap could provide nearly all the energy required. The battery capacity also provides a time buffer in case an ROV system failure or bad weather prevents dives. The Deep-ESP can continue to run normally for about 72 hours from the last sled swap.

With the short and unpredictable weather window at Axial seamount, we wanted to ensure the recovery of the Deep-ESP lander via an acoustic signal if the deployment needed to be called short, or if the ROV was unable to dive. However, a significant risk arises when the lander is connected to the ROV or battery sled. Although this underwater connector can be ROV coupled and released, it must be pulled directly inline. As the battery sled weight is much more than the buoyant force of the released lander, the sled could prevent the lander from ascending to the surface. This would leave the lander tenuously leashed 5 meters above the sled, making an ROV dive to decouple it a risk to the ROV tether. Additionally, there is no assurance that this coupling would stay connected and the lander might come free some weeks or months later, possibly when severe winter weather would ensure its destruction on the surface.

The same risk arises if power was lost to the ROV while downloading data or charging the Deep-ESP; the coupling may not separate from the lander when manually recovering the ROV. The lander is not heavy enough to prevent an unpowered haul up of the ROV; however, there are many ways that this action would result in major damage or complete loss of the Deep-ESP, the ROV, or possibly both.

To mitigate this risk, we considered several methods of spring or battery powered coupling ejectors or cable cutters, however, we found a much simpler and more reliable solution. The coupling is normally in a frame on the sled so a single ROV manipulator can connect or disconnect the coupling. The underwater connector requires less than 90 N to separate if pulled directly in line, much less than the 450 N buoyant force

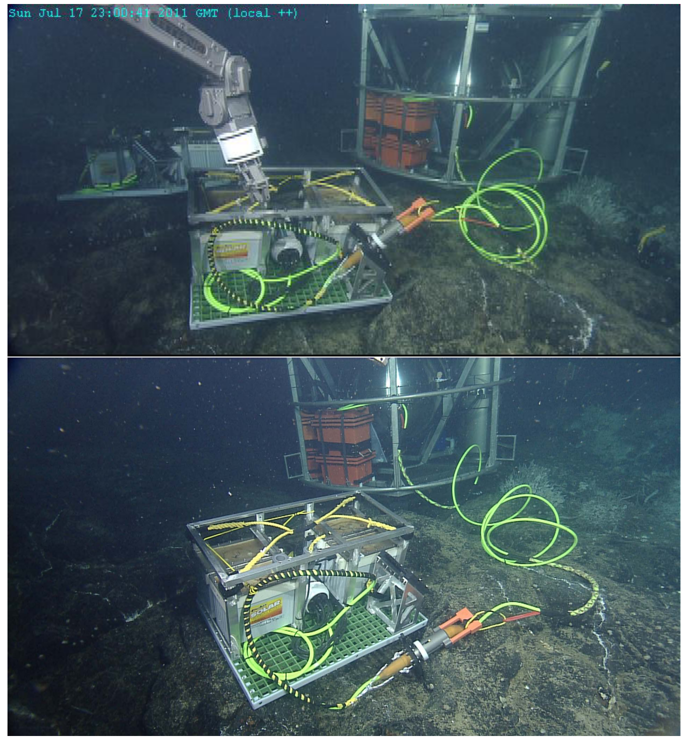

Fig. 4. Deep ESP deployed at the ASHES vent field within the Axial seamount caldera. (Top) An auxiliary battery pack is being repositioned by the ROV Doc Ricketts; (Bottom) The underwater coupling to the battery sled is removed from the sled and laid on the seafloor, ensuring in-line separation if the lander is released.

of the ascending lander. The cable exits the connector at an angle and the coupling frame on the sled also sits at an angle, making a direct inline pull on the coupling improbable. However, by leashing the coupling handle to cable so that cable tension pulls in-line with the connector and removing the coupling from the frame to lie freely on the seafloor, we mitigated this risk (Fig. 5). This placement frees the coupling to be lifted and align with the cable tension, allowing the coupling to be pulled apart, thereby separating the equipment.

VIII. Axial Seamount Deployment

Axial Seamount is an active underwater volcano ~470km west of the Oregon Coast with a recorded history of eruptions. The most recent eruption was in 2011, only months before Deep-ESP operations in that area. In the southwest part of the caldera floor, nearly 1600m deep, is a diffuse vent field known as ASHES (Axial Seamount Hydrothermal Emissions Study), which is a future site for cabled instrumentation via the Ocean Observing Initiatives (OOI) Regional Cabled Observatory.

In July of 2011, we deployed the Deep-ESP at ASHES to determine if microbial populations from diffuse flow vents could be distinguished from those living in the surrounding benthic water autonomously, in real-time. Diffuse flows are typically mixed and cooler than black smokers or chimneys, <100°C. Sulfide mounds, bacterial mats and macrofauna can be common around these slow discharges [8].

The lander was dropped onto a smooth lava flow, ~150m northeast of the field. Using MBARI's ROV Doc Ricketts, a suitable vent was found and the Deep-ESP repositioned to a spot about 10m from the vent. The wand system was deployed, permitting sampling from both the diffuse flow vent and the ambient water at the lander.

Over five days the Deep-ESP collected several water samples from both the vent and the ambient water around the lander. Some samples were processed immediately by the instrument, but many samples were also archived by the Deep-ESP for further analysis in the lab. The context sensors continuously recorded the physical conditions of the water being drawn by the wand. The ROV was used to swap the battery sleds, which got recharged nightly aboard the ship, but also to plug in directly to the Deep-ESP to have a much faster data connection and recharge the lander's batteries when needed. With the battery arrangement we were able to devote significant ROV time to other research around the vents, rather than just powering the Deep-ESP. The ROV was not operational for two days during the deployment, but the onboard batteries and battery sled allowed the Deep-ESP mission to be completed with minimal disruption.

The contextual sensors on the wand and lander showed significant difference in water properties being taken into the instrument for analysis, indicative of the different source waters (Fig. 5A). Real-time quantitative polymerase chain reaction, qPCR, performed *in-situ* by this system shows a difference in 16S gene abundance between the two sources (Fig. 5B) and *in-situ* sandwich hybridization assay (a direct detection method of ribosomal RNA with no amplification) also shows different community structures between the vent and ambient water sources (Fig. 5C).

IX. Conclusion

A system for real-time *in*-situ microbial analysis and archiving, including source water physical and chemical context monitoring, has been developed for use to 4000m ocean depth. The Deep-ESP is an evolution of technology that brings real-time *in situ* microbial analysis to new environments by developing environment interface equipment so a core detection instrument can function under extreme conditions. Other versions of the ESP are deployed on shallow water moorings, open ocean drifting platforms, and on piers above the sea level.

The Deep-ESP has been successfully deployed several times for durations ranging from a few days to a few months in various parts of the Pacific Ocean to accomplish a number of different scientific goals. At the time of this writing, the Deep-ESP is deployed and operating on the Monterey Accelerated Research System (MARS) in Monterey Bay [9], and will complete a six month deployment in October 2013. Future developments of the Deep-ESP will include integration of additional contextual sensors and adaptive sampling, where an ESP microbial sampling and processing event can be autonomously initiated when contextual sensors detect specific environmental conditions or patterns.

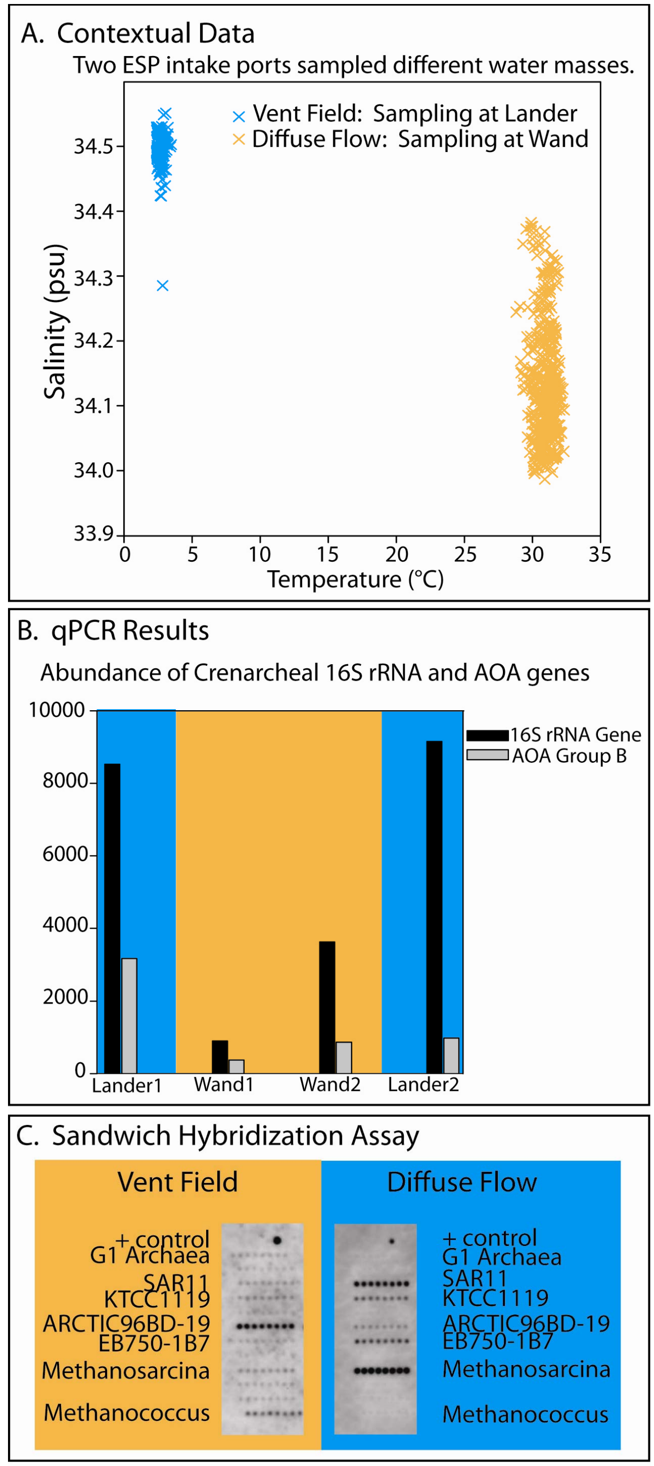

Fig. 5. Data obtained while operating the Deep-ESP at ASHES. (A) Temperature/salinity plots from context sensors show samples collected are from two distinct sources; (B) Real-time qPCR shows differences in gene copy number (Group 1A and AOAb genes) depending on collection site; (C) Sandwich hybridization shows differences in rRNA community structure when sampling away from or within the vent.

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