Lung Inspired Deep Water Sampling Module

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***Abstract*— A robotic system that can perform *in situ* microbiological identification and quantification of larvae, bacteria and other microscopic organisms at depths up to 4000m has been developed and deployed by the Monterey Bay Aquarium Research Institute. The system is composed of a low pressure detection instrument and a Deep Water Sampling Module that collects up to 10L of seawater and decompresses it for analysis. The architecture for the Deep Water Sampling Module was inspired by lungs and the mechanics of breathing.**

***Index Terms*— Hydraulic systems, Pressure control, Remote sensing, Sea**

I. Introduction

COLLECTING scientific information about life in the deep sea is challenging both technically and logistically, due to pressure, temperature and remote access issues. A mission of the Monterey Bay Aquarium Research Institute (MBARI) is to advance our understanding of the oceans through technological innovation. One such innovation is the autonomous robotic device, the Environmental Sampling Processor (ESP), which functions as a microbiology laboratory for *in situ* molecular identification and quantification of larvae, bacteria and other microscopic organisms in near real time [1], [2]. Collection and analysis *in situ* avoids sample alteration and the time lag that can occur when samples are sequestered and transported to the laboratory for analysis. Additionally, as a robotic system, the ESP can be deployed for months, tracking microbial activity over time without the need for human presence.

When deployed in the ocean, the ESP rests inside a pressure housing with valves to the ambient seawater. During sampling, the ESP opens these valves and filters a volume of seawater, concentrating microbes for autonomous detection by means of a luminescent sandwich hybridization assay [3] and real-time quantitative PCR [4], or preserves samples for later analysis [5]. Because the air pressure within the ESP housing is at one atmosphere and the seawater fluidic path through the ESP instrument is only rated to 500kPa, the basic system is limited to a 50m operating depth.

A science goal at MBARI is to enable the same *in situ* microbiology research capability in the deep ocean (>50m to 4000m) where the pressure can be 41 MPa. Rather than redesigning the fluidic path through the ESP itself, which would have been cost and time prohibitive, we chose a more expedient approach; to develop a pressure-modifying interface that could feed reduced pressure seawater to the instrument. The decompression interface idea evolved from a 1000m depth version to a 4000m version, called the Deep Water Sampling Module (DWSM). We studied several concepts for the DWSM, evaluating their potential to best meet the science objectives, finally selecting a differential pressure driven system inspired by lungs and the biological process of breathing. System design and fabrication found many parallels to lung structures and processes of inhaling and exhaling seawater, even to the point it garnered the nickname, “aqua-lung.”

II. Deep Water Sampling Module

MBARI developed and tested the first pressure modifying deep ESP system to1000m, which used linear actuated cylinder and piston assemblies to collect and decompress seawater. One large cylinder was used to inhale and sequester the seawater, and another much smaller one to decompress the sequestered seawater. Once decompressed to a safe pressure range, the system would open valves to the ESP instrument; filter the water, passing the filtrate to the back side of the sample piston. After sampling, the ESP was valve isolated, and the cylinders recompressed to ambient pressure. Although the cylinder and piston method of the 1000m system appeared a straight forward approach to sequestering and decompressing *in situ*, many of the decisions in the DWSM development were based upon lessons learned from the 1000m system.

A. Larger Sample

In the deep ocean, microbial life can be much less abundant than at the surface. The 1000m interface could only sequester 1.8L of seawater, which was found to be insufficient for all the science goals. A 10L sample of raw seawater was estimated to be necessary, but a drawback to the linear actuator and piston method was that the size of the mechanism is proportional to the volume sampled. The length of the full piston and linear actuator assembly was 1.15m. Increasing the sample volume of that diameter piston to 10L would have required a mechanism

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over 6m long, intractable to build and deploy into the ocean due to the size and weight of hardware required. Alternatively, a larger diameter cylinder could be used, but would have required over five times the piston surface area, higher capacity motor controllers and power supplies, and heavier actuators and pressure housings.

B. Active dynamic control.

The 1000m system required that linear actuators on both the sample and decompress pistons be powered and maintain position control at all times in order to maintain the decompressed state. If power was lost or the controller faulted, sea pressure could back drive the linear actuator and piston, returning the sequestered volume to ambient pressure very quickly, likely damaging the ESP instrument. To prevent this risk, we set a design goal that the DWSM maintain an instrument safe state in the event of any single mode failure.

C. Testable Design

One development complexity with the 1000m Deep ESP system was that it could not be tested on shore under the pressures it would experience during deployment. The system needed to be fully immersed in the pressure environment for the forces to be exerted during decompression, and it was much too large to fit in any readily available hyperbaric pressure chambers. This made actual deployments in the ocean the only place that engineering testing could be done. It took several months of test dives and iterative modifications to resolve a pressure induced control instability with the linear actuators and fix leaking valves and seals. To prevent similar development problems in the DWSM, we set a design goal that the system be testable at MBARI, at deployment pressures.

III. Design Process

With these lessons learned, and continual feedback from science users, we determined general system requirements, functional requirements and design goals. The DWSM would need to sequester up to 10L of seawater; decompress that from a potential 41 MPa to 300 kPa absolute, provide the sampled water to the core ESP for processing; collect the filtered water back from the core ESP; and prepare for the next sampling event. The functional requirements and design goals were as follows:

a. Operate from 0 to 4000m
b. Collect up to 10L of seawater
c. Sequester a single volume for processing
d. Maintain a homogenous sample
e. Prevent sample-to-sample contamination
f. Deployment for up to 1 year duration
g. Operate in water temperatures, 0-35˚C
h. Function by a series of stable, safe state sequences
i. Deployment pressure testable while on shore

A. Conceptual Design

A systems engineering approach was used to develop the sequence of operations that the DWSM would perform, regardless of hardware implementation. The DWSM provides the ESP with a volume of water to sample and process via a series of steps, each transitioning from one stable condition to another stable condition, so that a single mode failure at any state would either remain stable at that condition, or predictably drift to the last stable condition. The sequence of steps is:

1. Intake a volume of seawater, sequestering up to 10L in the sample bag
2. Isolate sea water valves
3. Decompress DWSM
4. If gas is generated from the sampled seawater, transfer and vent off; resume decompression
5. Once stable decompression is reached, isolate oil valves.
6. Open water isolation valves to the ESP core
7. Push water from the sample bag through the filtration stage of the ESP, collecting the filtered water in the exhaust bag
8. Once filtering of the sequestered sample is complete, close isolation valves to the core ESP
9. Recompress the system to ambient pressure
10. Exhaust water, and prepare for next sampling event

B. Lung Based System

Several different design concepts were formulated, compared and evaluated for the DWSM. The concept with the greatest potential for meeting the requirements and design goals was one modeled after mammalian lungs and the biological processes of breathing.

To inhale air into the lungs, the human body generates reduced pressure inside the chest in two ways. Most notably, muscles of the diaphragm contract, increasing chest volume. Also, accessory muscles in the chest lift the ribs upward to additionally expand the volume. When an air passage is open via the mouth or nose, the ambient air pressure drives air in the lungs, filling the volume made available. To exhale, the diaphragm and accessory muscles relax and the weight of the chest places the air inside the lungs at higher pressure than ambient. To exhale more forcefully, abdominal muscles contract to push upward on the diaphragm, increasing the pressure more, and air is forced out.

The DWSM fills and empties seawater bags in the same way that lungs inhale and exhale air. DWSM consists of two similar two-story tower assemblies each 1.5m tall, labeled Sample and Exhaust (Fig. 1.) Each tower consists of a central bulkhead plate sandwiched between identical pressure housings. Each top housing is a one-atmosphere dry chamber that contains control electronics, actuated valves and pump motors to direct the water and oil. Each bottom housing is a variable pressure oil filled chamber, containing a flexible bag for holding the seawater, as well as pumps and monitoring sensors. Outside of these towers is an external oil reservoir at ambient seawater pressure.

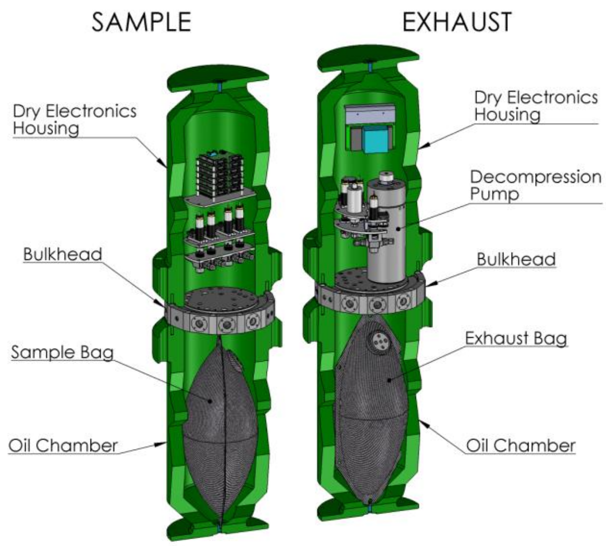

Fig. 1 Cutaway view of the DWSM tower architecture, showing pressure chambers, bulkhead plates, and major components.

The DWSM mimics human breathing action by pumping oil into or out of the bottom housings containing the sample bags to generate a pressure differential with the sea. This differential pressure is transferred through the flexible “lung” membrane bags to the raw seawater, which then flows if allowed to. The raw seawater is drawn into the system, decompressed, pumped thru the ESP, and exhausted back to the ocean all by pumping hydraulic fluid into or out of the oil chamber (Fig 2).

A major benefit of oil pumping systems, instead of piston cylinders like the 1000m version, is the pumps contact only oil, effectively eliminating pump corrosion and contamination concerns. Additionally, the DWSM pump design decouples the sample volume from the size of the driving mechanisms. The pumps can move greater volumes by simply allowing more pumping time. The sample volume could be increased by using larger chambers and bags, without significant alteration of the driving mechanisms.

The DWSM uses three oil pumps to move the seawater around and decompress the system. The bidirectional Intake/Exhaust Pump (IEP) is used to generate low pressure differentials to ambient, and is used to fill and empty the seawater bags. To create the decompressed environment for the ESP to filter, a unidirectional decompression pump is used to pump oil out to ambient storage at high pressure differential. A third Sample Pump (SP) is used to pressurize one bag versus the other and thereby drive water through the ESP filter stage.

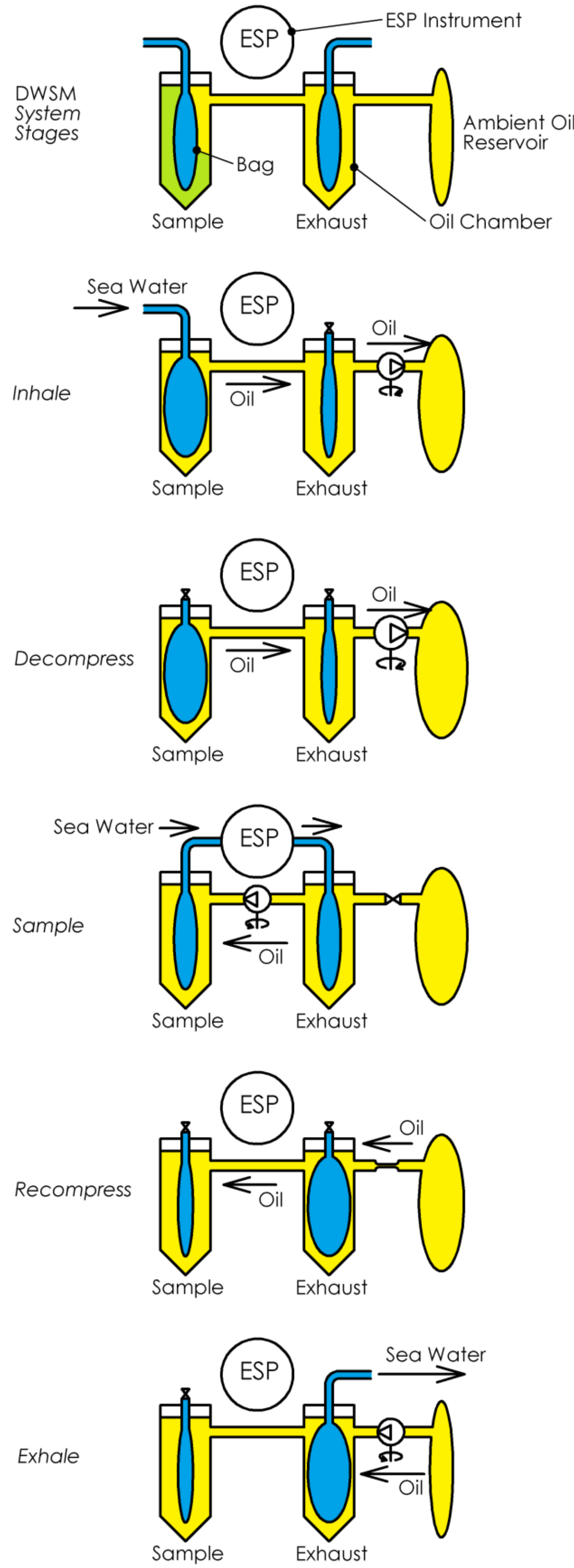

Fig. 2 Simplified schematic of DWSM system showing the stages of inhaling seawater, decompressing, sampling by the ESP instrument, recompressing and exhaling the filtered seawater

Inhale/Exhale

When inhaling seawater, oil is pumped from inside the oil chambers to a storage reservoir outside at ambient pressure outside of the DWSM. This pumping decreases the pressure inside the chambers and the higher ambient pressure forces seawater to flow into the selected valve port, filling the appropriate seawater bag. In exhaling, oil is pumped into the chambers from the reservoir, increasing the pressure inside the chambers above ambient. The seawater in the appropriate bag is pushed out through the selected valve port.

Decompression

Compared to the other concepts considered, a major benefit of the lung inspired system is that it functions by pressure differentials already. Decompression is the same action as inhaling, pumping oil out of the hard volume. With the intake valve closed, another pump of much higher pressure capacity is used to pump oil from inside the chambers to the ambient storage. As there is no open port for the seawater to make up the pressure differential, the pressure inside the chambers continues to drop until the target pressure is reached.

Sampling

After decompression, a flow path is opened through the ESP instrument's filtering stage. The sample pump pumps oil from the exhaust chamber to the sample, slightly pressurizing the sample bag relative to the other. This drives the raw seawater through the filter membrane in the ESP instrument, and into the bag of the exhaust chamber.

Recompression

After the filtering is completed, the ESP isolation valves are closed and the DWSM chambers can be brought back to ambient pressure safely. Recompression is accomplished by allowing oil from the ambient reservoir to flood back into the decompressed chambers. To prevent a potentially damaging pressure spike, the decompressed volume is initially opened thru a restricted flow needle valve, adding significant flow resistance. When the pressure differential between the chambers and the sea is small, a full port valve is then opened to the ambient storage so that the pressures may finish equalizing much more quickly.

The decompression pump can only pump oil out of the chamber, so the restricted flow valve allows for a method of slightly increasing the chamber pressure when decompressed. So, if the target pressure were overshot during decompression (e.g. the DWSM chambers were brought down to an absolute pressure of 150 kPa instead of the desired 300kPa), it can be brought up slightly by momentarily allowing oil flow through the restricted flow valve.

Proof of Concept

The bulkhead plates of the final design would be complex machined manifolds that all equipment converges into, so changes to the flow paths could not be made without significant redesign and fabrication costs. Prior to starting detailed design, a proof-of-concept was fabricated out of PVC piping, valves, and IV bags that could inhale, decompress, sample and exhaust 2L of water. This proof-of-concept was used to demonstrate the system, verify the sequence of operations, and most importantly, lock down the valve scheme for detailed design.

IV. Detailed Designs

There are many different components and subsystems to the DWSM, however a few key ones enable this technology and shall be presented in greater detail.

Oil

The hydraulic fluid used in DWSM is nontoxic silicone oil, 20 cSt polydimethylsiloxane, and was chosen as it is inert to the ESP detection chemistry in the event trace amounts get into the sampled seawater. A benefit to the silicone oil is that it is extremely hydrophobic and lighter than water. In the event of a seawater leak into the oil, the seawater will collect in the bottom of the oil chambers and not mix or foul the oil, which would damage the pumps. One drawback of the silicone oil is that it will affect most silicone rubber materials in contact with it, including seals and orings. Thus, nitrile materials were selected whenever possible and COTS components were carefully reviewed to ensure that no silicone materials were exposed to the oil.

Intake/Exhaust Pump

To inhale, the DWSM sets oil valves to allow free oil flow between the Sample and Exhaust chambers and to pump oil to the ambient storage with the Intake/Exhaust pump (IEP). A seawater flow path is opened to one bag, and the seawater is inhaled as oil is pumped out. Exhaling is the same process in reverse, with the IEP pumping oil into the DWSM chambers.

The IEP resides in the Exhaust oil chamber and pumps oil to and from the ambient oil reservoir. The pump body is fully submerged in the oil so that even under high ambient pressure, the pump only needs to generate a relatively low differential pressure, around 200 kPa, to drive inhalation and exhalation. Although the pump is located in the oil, it is magnetically coupled to a motor and encoder in the dry electrical housing on the other side of the bulkhead plate.

In order to control the amount of seawater inhaled in the bags, the system takes advantage of the 1-to-1 relation of inhaled seawater to pumped oil. To have 10L of seawater in the sample bag, 10L of oil is pumped out to ambient. The IEP is a positive displacement, valveless rotating piston pump, with good dosing accuracy. The volume of seawater inhaled is controlled by tracking the motor encoder and controlling the number of revolutions of the IEP.

Decompression Pump

The decompression action is made by valve isolating the DWSM chambers from the sea, allowing free oil flow between the Sample and Exhaust chambers, and then pumping oil from inside the Exhaust chamber to the ambient storage outside.

About 1.5L of oil needs to be pumped out to decompress the DWSM from 41 Mpa to 300 kPa absolute. Water is slightly compressible and is about 2% denser at 4000m than at the surface. The silicone oil is similarly compressible. In addition to pumping the volume needed to expand the seawater and oil, the decompression action also needs to pump out the volume that the pressure housings lose when they deform under the external ambient pressure.

As the pressure inside the chambers drop, the decompression pump needs to generate greater differential pressure to pump oil out to the ambient storage, eventually needing to generate pressure equivalent to the ambient sea pressure. The decompression pump is a three piston, cam driven, high pressure hydraulic pump coupled to an oil compensated 500W brushless DC motor. The pump motor is driven by a motor controller in the dry electronics housing. The four conductors for the brushless motor are passed into the oil chamber by a pressure rated connector, but the motor's hall sensor and encoder are located in the dry electronics housing, magnetically coupled to the backside of the rotor shaft. This magnetic coupling not only keeps the encoder out of the oil, but allows for a smaller pressure connector to the motor, saving valuable space on the bulkhead plate.

Sample Pump

After decompression the DWSM begins the sampling process. The DWSM opens water valves to the ESP instrument, and the sample pump is used to generate a differential pressure between the Sample and Exhaust chambers. The differential pressure drives seawater through the filter membrane within the ESP. The speed of the sample pump is servo regulated to maintain the appropriate pressure differential as particulate matter builds up on the filter.

The sample pump is a valveless rotating piston pump, similar to the IEP, but of smaller displacement. It is submerged in the Sample side oil chamber, and is also magnetically coupled to a dry motor and encoder. The volume of seawater filtered is controlled by tracking the encoder of the pump. The Sample pump is bypassed during decompression and recompression, allowing free oil flow between the chambers. The bypass is also used to equalize chamber pressures, so the pressure servo can be zeroed prior to start of filtering.

Compliance

With the change to a reciprocating pump, the IEP induces pulsed flow that is marginally within limits of the magnetic coupling and the motor controller, but still results in undesirable vibration and motor heating conditions. A spring loaded pulse damper was developed to even out the oil and water flows. Typically pulse dampers use an air chamber to buffer the pressure, however, at 4000m depth, such air pockets disappear. This forced MBARI to develop a bi-directional spring loaded pulse damper, and is used on both the IEP and the Sample Pump. On the IEP, the spring loaded damper functions as a simple pulse damper, evening out the pressure spikes from the piston pump cycling. For the Sample Pump, it also functions as spring driven compliance, resulting in more steady pressure on the filter membrane.

Water Bag System

The DWSM holds the raw and filtered seawater in flexible bag assemblies suspended in the oil chambers. The bag assemblies consist of the bag itself, a multiport cap, internal tubing, a stirring pump, a pressure switch assembly, and a seawater isolation diaphragm for the pressure switches (Fig 3).

The 10L bag is made of woven nylon with a polyethylene lining, and is commercially manufactured for water storage while camping. The only modification is heat sealing the outer seam of the bag to prevent any shedding of nylon fibers into the process oil. Although there have been no failures of the bags, they are replaced after every major deployment.

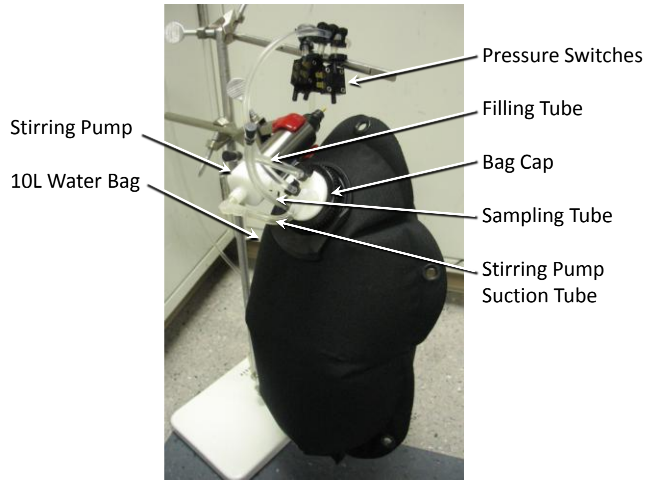

Fig. 3 DWSM 10L Seawater bag assembled and inflated with air for pressure switch testing.

The custom designed bag cap replaces the stock cap, and connects the seawater ports of the chamber to filling and sampling tubes that go into the bag. The filling tube opens to the very top of the bag, so that if any gas is present or generated during decompression, it will collect at the top and can be vented out. The ESP sampling tube pulls water from the 2/3 depth point in the bag. This depth point of the bag was chosen to avoid collecting any clumped sediment from the bottom, but to also reduce the likelihood of drawing air into the ESP instrument once filtering has started, which would prematurely stop filtration. The finer porosity (0.2 micron) filter membranes, used in the ESP for capturing bacteria, will not pass air once they become wet.

The seawater bags can suffer from the same problems as lungs; microbes and particulate can collect in the lower portions. In the DWSM, biological material must not settle out for two reasons. For the ESP instrument to accurately measure quantities of microbes per volume of seawater, the particles must remain in suspension. In addition, particulate that settles and is not expelled could contaminate future sampling and analysis. To keep the particulates suspended in the 4k DWSM bags, a stirring pump draws water from the bottom of the bag and returns it near the top.

Pressure switches

Filling the DWSM bags too much may cause a burst or seeping of seawater into the process oil. Conversely, an overly emptied bag can be squeeze damaged at the hard cap. During normal operation, the pumps may produce pressures that would easily damage the bags. Thus, we designed the DWSM with pressure switches to monitor the pressure differential across the bag membrane for three possible states. The first, a 'burst' condition, is when the bag is in danger of being overfilled and is signaled by the pressure in the bag being 3.7 kPa above the oil in the chamber. A second, 'squeeze' condition, is signaled when the bag pressure is 12.5 kPa below that of the oil. Both the 'burst' and 'squeeze' conditions signal to the control system, but also cut power to any pump in the direction that could cause damage. Pump power in the opposite direction is maintained so that the DWSM can recover from a 'burst' or 'squeeze' fault without recovering the deployed instrument. Empty, a third condition that is not a fault state, and is normally encountered with every exhalation cycle is signaled when the bag pressure is 3.7 kPa below the oil chamber. The control system manages this condition; shutting off the pump and setting the volume counter to zero, declaring the bag empty, and then continues with the planned mission.

The switches used have a double contact that is vibration and acceleration resistant. However the contacts are exposed to the working fluids and exposure to the seawater within the bags would always indicate a closed switch, regardless of the pressure state. To prevent this, the bag cap contains a small flexible diaphragm transfer barrier, filled with silicone oil. In this manner, the contacts of the pressure switch are exposed only to oil, but still reference the pressure in the bag.

Pressure boundary

All equipment in the DWSM is structurally mounted to the bulkhead plates (Fig. 4) which also function as the main fluidic manifolds and penetrator bases for cabling and fluid connections. The design of these plates became the focal point that all the other components and system designs keyed into, but where they also competed for space. Additionally, these plates are part of the pressure boundary to the electronics chamber, and have to hold back the 41 MPa pressure when the oil chamber is at ambient seawater pressure. To complete the design of these plates, many of the other system components had to essentially be designed simultaneously. The plates are made from 6Al4V titanium for strength and corrosion resistance as they do manifold the raw seawater.

Fig. 4 Sample and Exhaust bulkhead plates for DWSM, made from 6Al4V titanium, Exhaust plate on right. Over 100 hours of CNC machining was required, and helical mill threading was used for the 80 threaded holes.

Bell shaped pressure housings are used for the oil chamber and the dry electronics chambers. The bottom (oil) chamber housing is bolted to the bulkhead plate, so that it can contain pressures greater than ambient when the system is exhaling sea water. The top housing (electronics) bolts to the bottom housing, sandwiching the bulkhead plate between them. For manufacturing convenience, the bell housings are all identical, and are currently made of anodized 6061-T6 aluminum for cost effective development. For yearlong deployments, the aluminum bell housings could be replaced with titanium ones without affecting the action or capabilities of the DWSM.

Part of the DWSM pressure barrier is the isolation shroud of the magnetic couplings. The MBARI designed isolation shroud of this magnetic coupling is made of 6Al4V titanium, and can be pressurized from either direction to 41 MPa. These 100mNm cylindrical magnetic couplings are used on the IEP and sample pumps to couple to the motors, and the decompression motor to couple to the encoder and hall sensor.

The high torque needed by the decompression pump is much greater than can be magnetically coupled, so its motor is hard coupled to the pump and oil compensated at the same pressure as the oil chamber. The motor resides in a 17-4 SS and 6Al4V titanium two-piece pressure housing that extends from the Exhaust bulkhead into the dry electronics housing of the Exhaust tower, and can be seen in Fig. 1.

Rotary Valves

The oil and seawater paths of the DWSM chambers need to be isolated from the sea pressure. Several high pressure 3-way valves are used in the oil and seawater flow paths. The three way trunnion valves are each attached to a motorized actuator and encoder for the ESP to automatically set the valve positions needed for each cycle of the sampling process. Three of these valves manage oil flow paths, and four valves for the seawater paths. As all these valves are stainless steel, eventually corrosion will require rebuilding the seawater valve system. For ease of maintenance, all the seawater valves are located in the Sample side dry pressure housing.

Pressure transducers

The oil pressures of both the sample and exhaust chambers are monitored by pressure transducers reading absolute pressure to 41 MPa. These transducers are also the source signal for decompression and the sample pump servo controls, typically around 300kPa absolute. However, that is low in the transducer operating range, and temperature changes can cause significant drift. A digital pressure transducer, located in the exhaust side, is used to calibrate the analog transducers (and the custom electronics connected to them) to verify they are reading accurately enough to open the bulkhead valves to the core ESP safely.

Electrical

The DWSM module works as an ancillary device to the core ESP, with a common design for valve motors and encoders. Because of these similarities, we decided to follow the blueprint of the core ESP and designed DWSM electronics similarly, simplifying part acquisition and communication between the two devices. The distributed control architecture consists of a top-level, non-real-time processor managing several small, distributed, real-time processors, each tasked with monitoring local state or function (e.g., pump state, valve positions). The top-level processor is part of the ESP instrument and performs coordination and sequencing to the distributed controllers [6]. The distributed controllers perform A/D conversion, and provide DC motor control for two servo channels and four rotary valves, as well as eight solenoid drivers. The core ESP instrument commands the DWSM by an RS-232 serial link, while the controllers within DWSM communicate on an I2C interface.

Unfortunately, this electronics arrangement did not support sensing the pressure state of the sample bags, i.e. the bag pressure switches. Thus, a new electronics board was developed that monitors DWSM pressure transducers and the pressure switch conditions of the bags. This new board provides a high precision analog signal over a large dynamic range (41 MPa to 300 kPa absolute) for pump servo control using pressure inputs from both the sample and the exhaust chamber transducers. This new board also signals the distributed controller of the normally encountered bag “empty” state, resetting the bag volume counter to zero. In the event of a “squeeze” or “burst” error, the new board latches the state and locks out pump power on the distributed controllers, preventing further pump operation that could result in bag damage.

V. High Pressure Testing

As earlier mentioned, a design goal was to be able to operate the system at deployment pressures while still in the lab. This goal put preference on DWSM concepts that could be pressure tested more easily. The architecture of the lung-based DWSM is such that all the operating pumps and motors are inside the pressure housings. There are only two ports that connect to the outside pressure, one for seawater and one for oil to/from the ambient storage. To test the DWSM at elevated pressure, only three things are needed, a source of high pressure water, a source of high pressure oil, and a mechanical bolting arrangement to hold the pressure housings sealed while pressurized internally. The pressure housings were designed to not only withstand the 41MPa of external pressure when deployed, but also to contain the same pressure internally when tested in the lab. For the bell housings, this required designing a significant flange and bolting assembly as well as a 41 MPa seal that functions with pressure from either direction. Fig. 5 shows the system under 20 MPa testing. The twelve high tensile jacket bolts on each tower are used only during lab testing. As ambient sea pressure is sufficient to keep the pressure housings assembled when deployed, prior to deployment the jacket bolts are replaced with four smaller stainless steel fasteners without any disassembly of the DWSM towers.

Fig. 5 DWSM assembly with flanges and jacket bolts for 20 MPa pressure validation prior to deployment. The jacket bolts are pre-tensioned to maintain seal integrity when internally pressurized.

A High Pressure Interface (HPI) system was developed for development pressure testing of the DWSM. The core of the HPI is a 45 MPa transfer barrier accumulator that provides pressure balanced water and oil to the DWSM so that it can operate at deployment pressure in the lab, executing all the operations under the same loads as when deployed. As the deep ocean is cold, to further simulate the conditions while deployed, the fully assembled DWSM, ESP instrument and HPI are placed in a refrigerated shipping container and operated at 2˚C. In this manner, all the pumps, switches, and valves operate the same sequences, at the same pressure and temperature as they will during deployment. Since the HPI pressure and cold test can be accomplished in just a couple days, it has become standard to do a full system validation at deployment temperature and pressure prior to every mission at sea.

VI. Deployments

The DWSM is deployed as part of the Deep ESP benthic instrument, which has been operated at several sea floor locations since April 2009. The Deep ESP assembly is a cylindrical package, 2m tall x 2m diameter, containing the DWSM, the ESP instrument, system batteries and context sensors. It has been operated autonomously and interactively via a remotely operated vehicle and cabled ocean observatory, the Monterey Accelerate Research Station [7].

In July 2010, the Deep ESP was operated at a methane mound in the Santa Monica Basin, Fig. 6 [Ussler, et al., in prep.], and at the Ashes Vent Field on Axial Seamount in July 2011 [Girguis, et al., in prep].

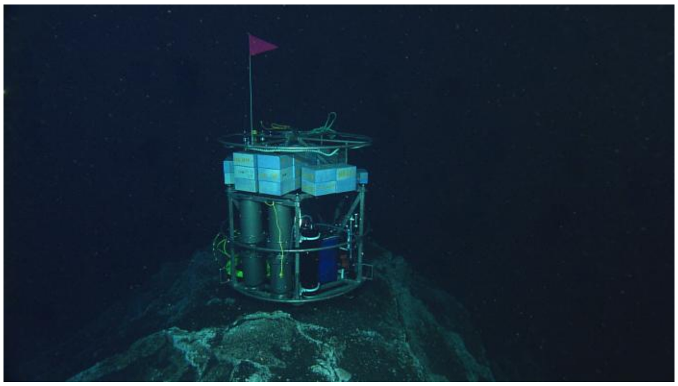

Fig. 6 Deep ESP system deployed on top the Southern California Methane Mound, 800m deep.

VII. Future Direction

This method of decompressing deep ocean water was the most expedient method of *in situ* microbial identification with the current detection technology, but it may not be ideal from a science perspective as the pressure state of the organisms is changed by the process. There is some concern that decompression may influence the microbial community structure, and thus the ESP may not accurately reflect the microbial populations in the environment. However, with the development of the DWSM and the HPI we can now consider methods of testing how the decompression affects the microbial populations that we wish to study.

The development of the DWSM continues and the next objective is a long term deployment on a cabled ocean observatory. As the DWSM bags and fluidics are exposed to raw seawater, after a period of time, flushing of the bags may not be enough to prevent microbial growth from contaminating the DWSM. As the ESP instrument can detect even just a few cells per liter of sea water, the need to clean and sterilize the bags and plumbing will likely become necessary. The capability of decontaminating the DWSM *in situ* was planned for in the original conceptual design, but has yet to be fully implemented.

Coupled with long term deployment capability is another science objective of the DWSM, to allow for *in situ* deep water incubation experiments. The concept of operation is that the DWSM would sequester a volume of seawater (either inside or outside of the chambers); add a volume of nutrient or other stimulus; and allow that mixture to incubate at the same temperature and pressure environment. This would enable research into microbial population or gene expression changes due to various environmental or nutrient stimuli.

Corrosion of DWSM components may become a greater concern for long term operations. Much of the complex components are titanium, but these can be a source for galvanic corrosion when immersed in seawater. We are planning studies to determine if a powered anode will be sufficient to protect against galvanic corrosion, as well as weighing the costs of additional titanium parts versus replacing expendable aluminum ones.

VIII. Conclusion

In order to significantly increase the depth capability of *in situ* autonomous microbial identification, an interface module was developed that can provide decompressed seawater for analysis by a low pressure scientific instrument at depths to 4000m. Lungs and the process of breathing inspired this Deep Water Sampling Module, enabling not only greater capacity, but a fault resistant system, and development testing under real pressure. The ESP with the DWSM is enabling new scientific research of the deep ocean with the goal of better understanding the life and chemistry of the deep sea.

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References

[1] Scholin, C, G. Doucette, S. Jensen, B. Roman, D. Pargett, R. Marin III, C. Preston, W. Jones, J. Feldman, C. Everlove, A. Harris, N. Alvarado, E. Massion, J. Birch, D. Greenfield, R. Vrijenhoek, C. Mikulski, K. Jones. "Remote detection of marine microbes, small invertebrates, harmful algae and biotoxins using the Environmental Sample Processor (ESP)" 2009, *Oceanography* 22:158-167.

[2] Environmental Sampling Processor website: http://www.mbari.org/esp/

[3] Greenfield, D., R. Marin III, G.J. Doucette, C. Mikulski, S. Jensen, B. Roman, N. Alvarado, C.A. Scholin. "Field applications of the second-generation Environmental Sample Processor (ESP) for remote detection of harmful algae: 2006-2007." 2008, *Limnology and Oceanography: Methods* 6: 667-679.

[4] Preston, C., A. Harris, J. Ryan, B. Roman, R. Marin III, S. Jensen, C. Everlove, J. Birch, J. Dzenitis, D. Pargett, M. Adachi, K. Turk, J. Zehr, C. Scholin. "Underwater application of quantitative PCR on an ocean mooring." 2011, *PLoS ONE* 6(8): e22522. http://www.plosone.org/article/info%3Adoi%2F10.1371%2Fjournal.pone.0022522

[5] Ottesen E, Robidart J, Preston, C. Scholin C., and E.F. DeLong 2011. "Metatranscriptomic analysis of autonomously collected and preserved marine bacterioplankton." 2011, *ISME Journal* epub: http://www.nature.com/ismej/journal/vaop/ncurrent/full/ismej201170a.html

[6] Roman, B., C. Scholin, S. Jensen, E. Massion, R, Marin III, C. Preston, D. Greenfield, W. Jones, and K. Wheeler, "Controlling a Robotic Marine Water Sampler with the Ruby Scripting Language" *Journal of the Association for Laboratory Automation*, Volume 12, Issue 1, February 2007, Pages 56-61

[7] Massion, E. and K. Raybould (2006). MARS, the Monterey Accelerated Research System. *Sea Technology*, 47: 39-42.