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Discussion Paper:

Emerging Technologies for Biological Sampling in the Ocean

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The objective of this paper is to present background information that will foster productive discussions on existing and emerging technologies in biological sampling. The paper presents examples of some biological sampling technologies that have been developed over the past decade, as well as emerging technologies that may be adapted for biological sampling. The focus is on collecting a physical specimen of an organism, regardless of the size of the sample. The paper concludes with some questions to guide biological sampling technology development in the context of a national ocean exploration program

The following assumptions form the basis for this paper:

1. There is a continued need for biological sampling in the context of a national ocean exploration program.
2. Existing biological samplers do not meet our projected needs.

Biological sampling in the context of a national ocean exploration program

A national ocean exploration program should incorporate documentation of living and non-living resources in the U.S. Exclusive Economic Zone (EEZ) and Extended Continental Shelf (ECS) and provide an initial knowledge base for hypothesis-based science and exploitation. The challenge is balancing the need to increase the scope of exploration with documenting

what one finds. The following questions should be addressed to guide biological sampling technology development in the context of ocean exploration, as well as to implement modifications to the current procedures, platforms, and tools as new technologies are developed:

- *How much information (e.g., data, samples, photographs) is needed to inventory living resources in the US EEZ and ECS?*

Is it important to know the number of species documented? This is a metric currently used by NOAA's Office of Ocean Exploration and Research for *Okeanos Explorer* expeditions. Implicit in this question is the understanding that an expert needs to verify the identity of the sample, or if new, to verify that it has not yet been described. There has been some debate about whether it is necessary to collect a sample to be able to identify it as a new species. A type specimen is highly desirable, but an illustration or photograph may be considered type material if it is impractical, unethical, or illegal to collect the organism (e.g., if it is rare, threatened, or endangered) (Winston 1999). A new species has to be thoroughly described. For some taxa, including many invertebrates that are found in the deep ocean (e.g., sponges, octocorals), this involves describing not only external morphological characteristics, but also microscopic examination and description of internal characteristics. A photograph will not suffice for new species identifications for such taxa.

- *How much information is needed to provide an initial knowledge base to generate hypotheses and/or to manage resources?*

Are maps, environmental data, photos, and videos an adequate knowledge base to support a robust grant proposal? For example, little is known about how organisms interact with each other or their environment in the deep ocean. Is it important to understand the functions of organisms and ecosystems to better understand the ecological services provided by living resources in the US EEZ and ECS?

Sampling the benthos

In oceanography, biological sampling is conducted mostly from remotely operated vehicles (ROVs) and human occupied vehicles (HOVs) that are outfitted with multifunction

manipulators and sample storage containers. Specialized tools for both ROVs and HOVs have been in use for decades for collection of biological samples, regardless of their size, shape, or consistency, whether they are sessile or motile, benthic or planktonic. There is one primary limitation related to the technology: not all manipulators are equipped to collect all types of biological samples, in particular, soft-bodied or fragile organisms and sediments for microbiology. For example, a claw is inadequate to collect a delicate anemone. Without a tool to match the properties of the sample, even highly skilled pilots have to spend additional time to collect delicate samples. New technologies could improve the efficiency of sampling benthic organisms, and continued developments of these technologies are expected to provide even more information.

Squishy fingers for fragile samples

Recent advances in the application of soft robotics technology have the potential to transform our ability to collect fragile samples. One application uses a composite of silicone with fiberglass and Kevlar fibers to make different shapes; the "squishy fingers" are pressurized with seawater to get the fingers to curl gently around a fragile sample (Figure 1) (Galloway et al. 2016). Future improvements to "squishy fingers" will incorporate *in situ* application of RNA stabilizers to samples at the time of collection to minimize changes in gene expression caused by stress during collection and transport of samples to the surface.

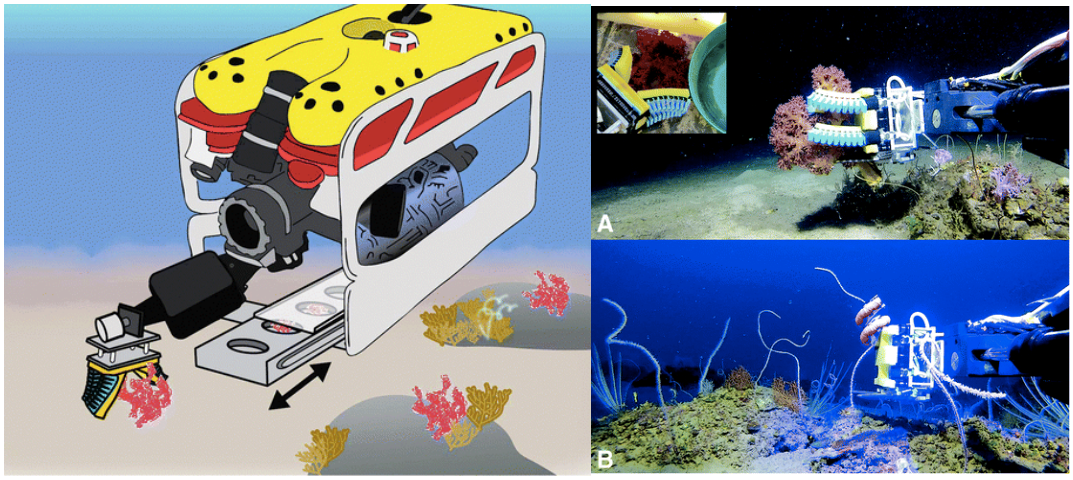

Figure 1. Left. Cartoon of ROV Seaeye Falcon ROV with soft robotic manipulator collecting a soft coral. Right. (A) Bellows-type gripper collecting a soft coral and (B) boa-type gripper collecting a sea whip at 100 m. From Galloway et al. 2016.

Beyond manipulator arms, hands, and fingers

Biological sampler and sensor technologies have progressed beyond manipulator arms and storage bins to incorporate advances in –omics, microelectromechanical systems (MEMS), and microfluidic technologies. They are already being used in coastal and ocean research, and innovative modifications based on these technologies are underway. Three examples are presented, as a basis for discussion of advances that could be made for deployment from autonomous ocean exploration platforms: Environmental DNA (or eDNA), the Environmental Sample Processor (ESP), and the Autonomous Microbial Genosensor (AMG).

Environmental DNA as a tool to assess community biodiversity

Over the past decade, DNA barcoding has emerged to complement and support the traditional identification of biological samples through morphological characteristics by using short genetic sequences to distinguish between species. It has been particularly useful in understanding biological diversity within and between ecosystems, with one important caveat: a reliable species database, with data on specimens that have been correctly identified, must be available (Vargas et al. 2015). Limitations to DNA barcoding and interpretation of barcoding data have been described (e.g., Collins & Cruickshank 2012). Although DNA barcoding still relies on having a physical sample, it has formed the basis for development of sampling technologies that require much smaller samples and are minimally or non-invasive, such as the Stinger needle-biopsy sampler (see below).

Analysis of environmental DNA (eDNA) has emerged as a potentially powerful approach to assess the diversity of biological communities. The premise is that marine organisms shed their DNA in the form of metabolic wastes, skin cells, and damaged tissues; analyzing the DNA can reveal what is (or has been) living in that environment. It has been particularly useful in assessing the diversity of vertebrates in nearshore environments (Port et al. 2016) and aquarium mesocosms (Kelly et al. 2014); a limitation is the need for a reference library of DNA against which to compare the DNA in the water samples. There are some significant technical issues that need to be addressed (Ausubel 2016, unpublished):

- There is no “best” sampling strategy. The volume of water filtered and the number

of samples analyzed will likely differ by site and species.

- Filters can get clogged, which will limit the volume of water that can be collected.
- Repeat DNA amplification of the same sample can give different results.
- Reference libraries for most species likely to be encountered in the deep ocean do not exist. And depending on the taxon, the gene sequences selected for analyses may not be able to differentiate between species.
- Ideally, eDNA should enable not only the presence or absence of an organism, but also its relative abundance. The challenge is assessing accuracy and sensitivity in natural settings, accounting for movement of water and animals, and understanding the roles of turbulence, salinity, pH, and other chemical variables on the rate of decay of DNA.

Eco-genomic samplers for *in situ* molecular analyses

Eco-genomic samplers and sensors detect molecular markers in the environment. As with eDNA, the assumption is that these markers can be used to identify specific organisms or genes. Sampler requirements include collecting, concentrating, preserving and lysing cells, applying reagents, removing particulates, and applying extraction chemistries (Scholin 2010). Eco-genomic samplers are designed to observe sets of defined biomolecular signatures in time-series, including sample archival for supporting discovery work post-deployment (Scholin 2013). Two examples are the Environmental Sample Processor (ESP) (Scholin 2010) and the Autonomous Microbial Genosensor (AMG) (Fries et al. 2007).

Both the ESP (Figure 2, left) and the AMG (Figure 2, right) are electromechanical/fluidic systems that collect water samples and robotically perform three actions: filter seawater, extract and partially purify RNA, and use molecular detection technology to identify microorganisms and their gene products *in situ*. Samples are preserved and stored for analysis after the instrument is recovered. A second generation of the ESP includes a CTD, an *in situ* nitrate analyzer, and an *in situ* mass spectrometer to measure flux between sediments & seawater. The major limitation of these systems for deployment on autonomous ocean exploration platforms is their size (Figure 2). Miniaturizing these

samplers so that they can be used on small AUVs could open up a new world for understanding deep ocean organisms and their communities.

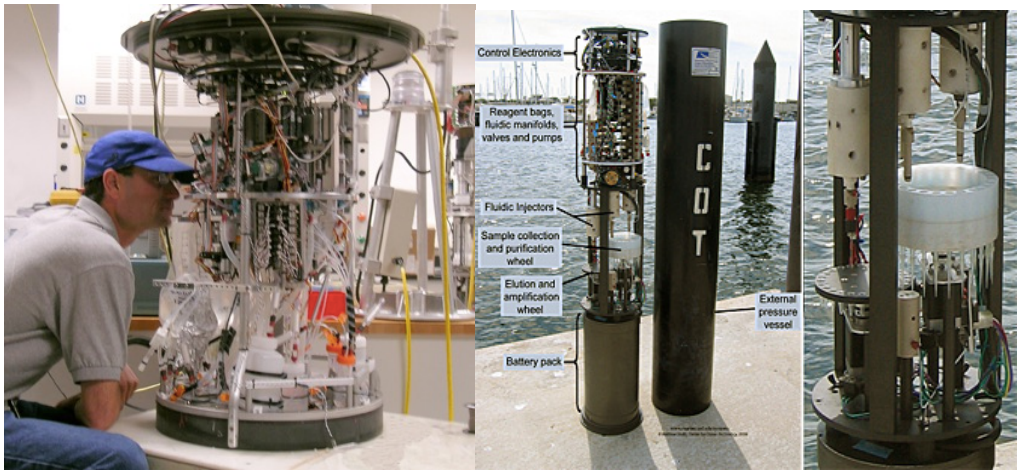

Figure 2. Left. Environmental Sample Processor (ESP). Right. Autonomous Microbial Genosensor. Photo credits: MBARI (left), University of South Florida (right).

The "Stinger" and "Mat Samplers"

The Mat Sampler (Figure 3) was developed for sampling microbial mats around hydrothermal vents (Breier et al. 2012). It allows for collection of multiple, discreet samples and can incorporate in situ physical, electrochemical, and optical sensors. The Stinger is a needle-biopsy sampler currently in development by CIOERT (Fries, Institute for Human and Machine Cognition). It incorporates design requirements gained from other underwater samplers, such as the Mat Sampler and samplers for chemical analysis of seawater (Fries et al. 2012). The purpose of the needle-biopsy sampler is to provide a minimally invasive way to collect, analyze and preserve samples of living organisms.

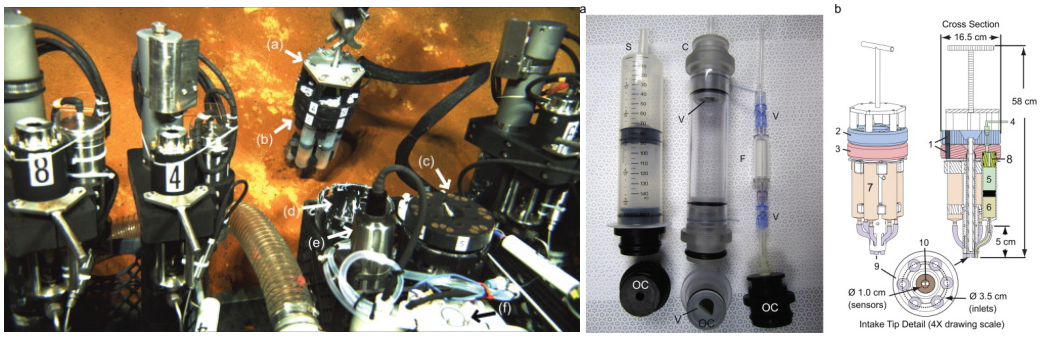

Figure 3. The Mat Sampler (left) deployed from the ROV *Jason*; right, sample can be collected in syringes, columns, and filters (Breier et al. 2012).

A different kind of water sampler – the "Plankzooka"

Sampling the vast midwater environment and the numerous fragile organisms that live there is particularly challenging. Cindy Van Dover (Duke University), Carl Kaiser (WHOI), and Craig Young (University of Oregon) developed an innovative concept for an AUV-deployed sampler that was designed and built by engineers at WHOI. The SentrY Precision Robotic Impeller Driven (SyPRID) sampler, also known as "Plankzooka", gently pumps large volumes of seawater through nets located within two carbon fiber composite tubes (Figure 3). On its maiden voyage in 2015, the National Deep Submergence Facility AUV *Sentry* carried SyPRID to more than 2,150 meters, where it sampled deep-sea larvae above a natural methane seep (Fischetti 2015).

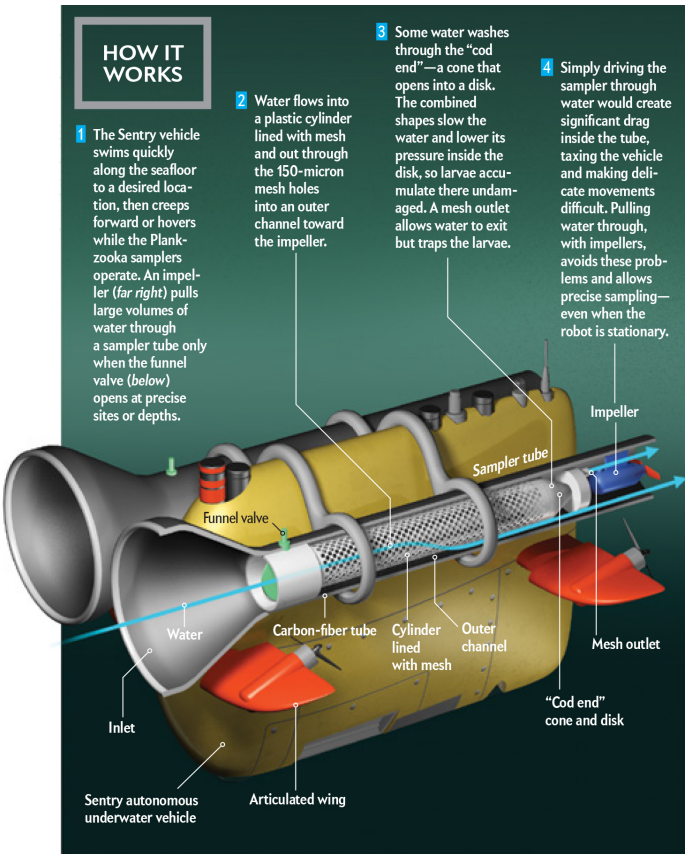

Figure 4. The SyPRID sampler. Illustration by Dan Foley, Scientific American, Nov. 2015. https://www.scientificamerican.com/article/meet-plankzooka-the-deep-sea-plankton-vacuum/

Specifications for the ideal biological sampler for a national ocean exploration program (recognizing the author's bias toward benthic sampling…)

- Small enough to meet payload restrictions of autonomous platforms
- Low power consumption
- Rapid sample collection (if dive time is a factor)
- Capability to collect multiple samples during a single platform deployment
- Minimally-invasive
- Capable of penetrating hard-bodied and soft-bodied benthic organisms and collecting sediment samples (for microbial samples)
- Sample can be transferred *in situ* to appropriate fixatives, to minimize post-collection changes
- Sampler can be "decontaminated" between sampling, to avoid carry-over of material from the previous collections
- A variety of fixative options are available, and can be selected at the time of collection.

"Lab-on-a-chip", microelectromechanical systems (MEMS), and microfluidics technologies have already been incorporated into the design of samplers such as the ESP, AMG, and Microbial Mat samplers, described above. As noted, these samplers need to be miniaturized to meet the payload requirements of the types of autonomous platforms currently envisioned for an expanded ocean exploration program. Emerging technologies in materials science, medical devices, microfluidics, and optics (to name just a few) will contribute to the development of the next generation of biological samplers in the ocean. Two examples are electroactive polymer technology (EAP), also known as "artificial muscle", for applications in ultra-low power, disposable, lightweight actuators, pumps, and valves (Chiba et al. 2011), and electric impedance microflow cytometry (EIMC) platforms—miniaturized flow-cytometers for *in situ*, non-invasive diagnostics (Du et al. 2013).

Our Forum co-chairs have guided us *"to creatively adapt and assemble existing technologies, and deploy them onboard autonomous devices, buoys, various so-called ships-of-opportunity and other platforms"* (Ausubel & Gaffney, 2016, unpublished, National Ocean Exploration Forum 2016, General Guidance). The goal of this paper is to provide some ideas for our discussion during the Forum. Moving forward, collaborations across scientific disciplines could lead to transformative technologies for ocean exploration.

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