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Modular Autonomous Biosampler (MAB)- A prototype system for distinct biological size-class sampling and preservation

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***Abstract*— Presently, there is a community wide deficiency in our ability to collect and preserve multiple size-class biologic samples across a broad spectrum of oceanographic platforms (e.g. AUVs, ROVs, and Ocean Observing System Nodes). This is particularly surprising in comparison to the level of instrumentation that now exists for acquiring physical and geophysical data (e.g. side-scan sonar, current profiles etc.), from these same platforms. We present our effort to develop a low-cost, high sample capacity modular, autonomous biological sampling device (MAB). The unit is designed for filtering and preserving 3 distinct biological size-classes (including bacteria), and is deployable in any aquatic setting from a variety of platform modalities (AUV, ROV, or mooring).**

***Index Terms*—AUV, ROV, mooring, size fractionation, filter system**

I. INTRODUCTION

The world's oceans contain vast unexplored regions and ecosystems that have yet to be characterized. The last 10-15 years have seen a tremendous growth in the development and application of new sensor platforms, specifically autonomous and Lagrangian platforms [17] such as Autonomous Underwater Vehicles (AUVs) [3], [2], [7], [12], drifters, and ocean gliders and especially in the realm of integrated Ocean Observing Systems (OOSs) [9] [6], [15], [13]. Each platform enables advanced sampling further into unique subsets of these uncharted regions. Nevertheless, a parallel growth in the realm of biosensors remains significantly behind that of geophysical sampling and biosampling is an under-represented sector in the implementation of OOS systems worldwide. As such, the potential for novel discovery and environmental health assessment from biological and chemical sensors is extremely high [14], [5], [4].

To date few biosampler systems have been developed to take advantage of the wide range of platforms available, most being limited to deployment on a single specific platform. This is particularly surprising in comparison to the level of instrumentation that now exists for acquiring physical and geophysical data (e.g. side-scan sonar, current profiles, suspended sediments etc.), in the oceans from autonomous and robotic platforms [1], [16] [19], [8]. Currently the most sophisticated biosampler that has been developed for marine systems is the Environmental Sample Processor (ESP) [10], [11]. The ESP has been designed to detect up to 49 pre-selected genetic targets (prokaryotic and eukaryotic) *in situ* and in real time [18]. This unit has been successfully deployed for extended periods on moorings in surface waters and in deep water from an ROV and is currently being developed for long-term deep-ocean observatory deployment. However, this highly engineered system, while very powerful for some applications, is extremely costly, requires *a priori* knowledge of the biological system, has limited sample capacity, and necessitates a significant maintenance schedule. In addition, its size limits deployment to larger platforms with reduced operating range. An example of another modern off the shelf biosampler is the McLane Research Laboratory Zooplankton Sampler (ZPS). This unit is designed solely for collection of zooplankton onto a single filter, has a limited 50-sample capacity, and has no sensor integration capability. In addition, the ZPS has a significant power requirement, and is of a significant size that precludes deployment on smaller AUV/Glider platforms.

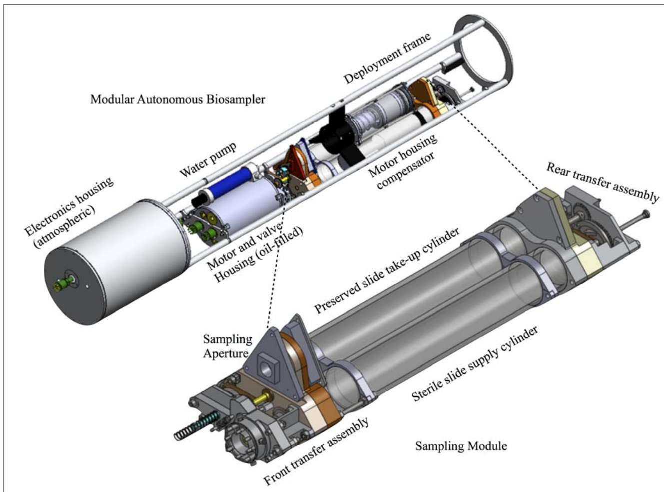

Fig. 1. Modular Autonomous Biosampler (MAB) Mark I System

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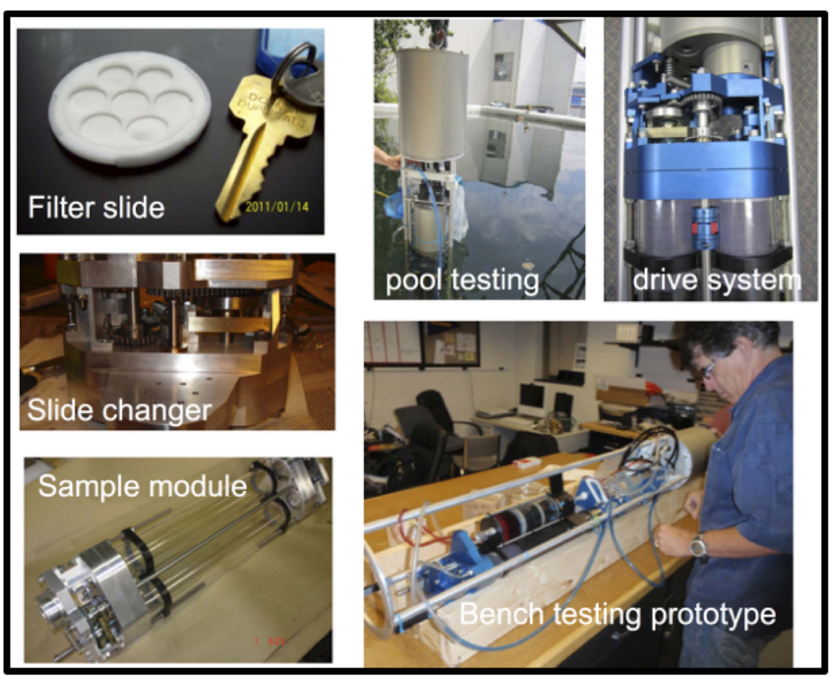
We designed and successfully developed a prototype modular autonomous biosampler (MAB) Fig. 1 that includes an innovative filter exchange system allowing 3 individual size classes from over 200 samples to be taken and preserved in a single deployment. Through our proof of concept project we jumped a major hurdle in complexity and solved a significant engineering problem- how to stack, transfer, and filter more than 200 samples for sequential collection and processing.

Here we present the overall design and integration of current-off-the-shelf technology into a novel configuration. While it is true that systems like the Hardy Continuous Plankton Sampler (circa 1930) have some historical bearing on this project and certainly point to the long standing need for a system such as the MAB, the development of a wholly new class of modular self-contained, programmable pump-filter systems is a significantly distinct undertaking. The development of this device is particularly urgent given the critical need in the community for biological sampling in connection with existing and planned ocean observatory and mooring networks and in response to time sensitive environmental issues like oil spills. Our goal has been the development of a compact, robust, cost-effective, high-sample capacity biological sampling system that can be deployed on a number of platform systems (i.e. AUVs, gliders, drifters, moorings, or ROVs). Our sampling philosophy is to collect samples that will be stored in any number of preservatives that allow long-term storage allowing both morphological and genetic (DNA and RNA) analysis at a home laboratory weeks or months later.

The oceanographic community is severely limited in the ability to conduct extended time series biological sampling in remote locations. This is particularly true for distant offshore and deep-sea sites. Our limited view of biological productivity in these locations comes either from opportunistic spot sampling or extrapolative remote sensing tools. The development of a low-cost high capacity smart biosampler that has the ability to store samples for extended periods of time for later analysis will satisfy the critical need to access these vastly understudied areas of our oceans. The ability to deploy such a unit on multiple platforms will meet the needs of a broad sector of the community currently developing access capabilities to these remote areas such as ocean observing networks and AUV/ROV platforms. The modular autonomous biosampler (MAB) system has the potential to dramatically increase our remote long-term biological surveillance capabilities.

II. System Description

In this project, a proof of concept biosampler was designed, built, and delivered Fig. 2. Benchtop testing of the prototype was conducted and a full assessment report, design diagrams, and a user-manual for the prototype system were developed. The prototype unit has allowed us to clarify and prove a number of the design features and while this prototype is presently too large to attach to a small AUV and not yet field-ready on its own, the lessons learned in the design and fabrication effort have provided invaluable guidance for an envisioned second generation version. Our invention consisted of a low-cost, high sample capacity modular, autonomous biological sampling system complete with control and sampling modules Fig. 1. The unit was designed for filtering



Prototype Modular Autonomous Biosampler	**Configuration**
Slide capacity	200
Slide diameter	49.5 mm
Depth capacity	200 m
Diameter x Length	229 x 1615 mm
Weight	35 kg
Power input	24VDC
Trigger Interface(s)	1. Interactive RS232 2. 5V rising edge
Temperature rating	0 to 35°C
No. of filter class modules	1

Fig. 2. The prototype MAB system is designed for (1) AUVs (2) ROVs and (3) ocean observing system moorings.

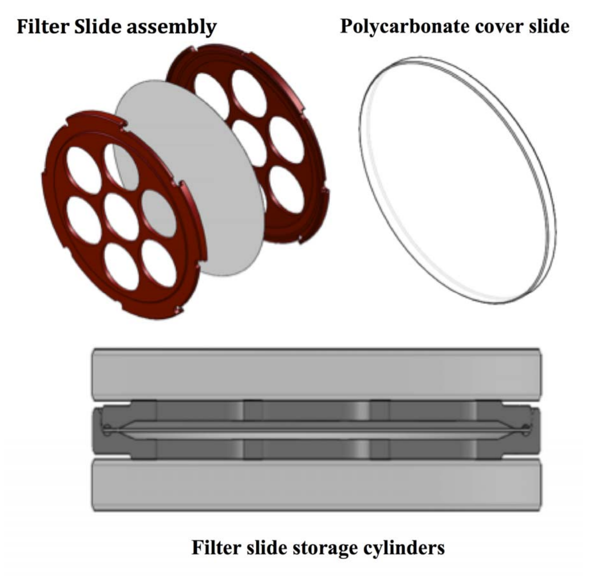


Fig. 3. Filter slide component including- slide cassette (upper left), cover slide (upper right) and stacked assembly (lower).

and preserving distinct biological size-classes (including bacteria) and is meant to be deployable in any aquatic setting from a variety of platform modalities (e.g. AUV, ROV, or mooring).

The following criteria have guided the design process throughout the development of the MAB system:

I. Modular design - allowing 1-3 sampling modules to be processed in series

II. Collect and preserve discrete biological samples ranging from zooplankton to bacteria for morphological and genetic analyses

III. High sample capacity- > 200+ samples

IV. Minimize cross-contamination and/or leaking between samples

V. Longevity - Goal to produce a full-ocean depth capable system that can operate autonomously for several months at a time

VI. Integration of off-the-shelf sensors (e.g. temperature, salinity, DO, Chl-a, etc.,) to enable “Smart” sampling capability, whereby the MAB will be triggered by ancillary sensor data.

VII. Deployable on a small person-portable AUV to demonstrate deployment capability on any platform

III. Results- Slide Exchange System

We sought to design and build a mechanism that would enable 3 size classes to be sampled simultaneously in a high throughput setting. This system was to allow discrete filters from three separate identical sampling modules to be loaded in series across a sample stream, so that up to three discrete size classes could be sampled from a single stream. The samples were to be isolated and stored in preservative. The system was to have the capacity to store at least 200 slide samples Fig. 3 per module, all within the confines of a 200 mm diameter cylinder (chosen to match the size requirements of small AUVs/gliders). This system was to demonstrate suitability for deployment on a mooring and on a small portable AUV. A patent application for the prototype system was filed in 2011 under application number 61/474,475. The prototype unit has allowed us to clarify and prove a number of the design features most important of which is that we demonstrated the ability to store and mechanically move and filter a large stack of sample slides (> 200).

The sampling modules were designed so that apertures from up to three separate modules could be stacked in series. With apertures locked together in this manner, the three modules would fit within a cylinder of diameter 230mm, 15% above the target of 200mm. The sample module was built to accommodate up to 200 filter cassettes Fig. 1.Standard 47 mm syringe filter membrane discs were chosen as our filter medium Fig. 3. We packaged these in custom 50 mm diameter by 2.5 mm thick sandwich cassettes (slides) with discs sandwiched between identical interlocking carriers Fig. 3. Consecutive slides are separated from each other by 50mm polycarbonate discs (cover slides), in order to prevent cross-contamination Fig. 3.

We limited production of the prototype system to one sampling module Fig. 2. A sampling module was designed to

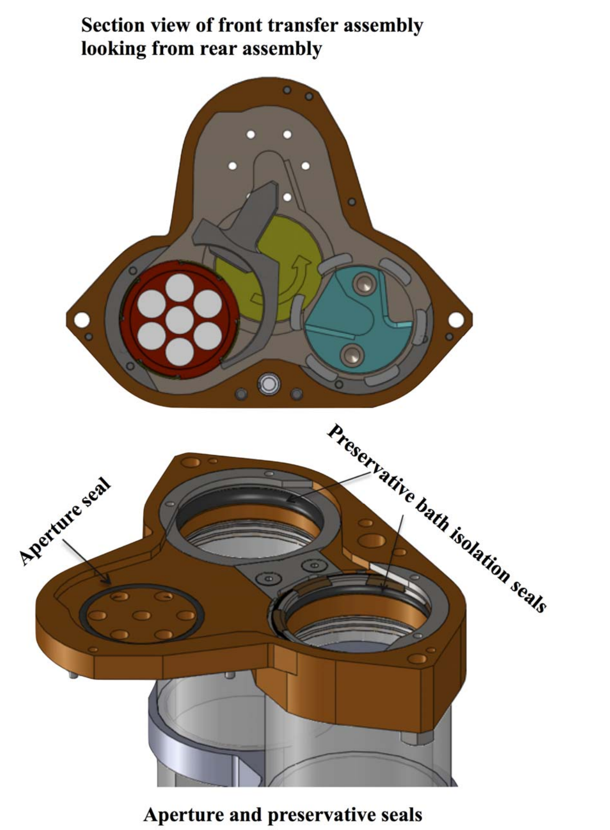


Fig. 4. Transfer assembly (upper), and filter aperture illustrating preservative bath seals (lower).

sequentially load filter cassettes from a sterile storage cylinder to a sample aperture Fig. 4 situated across a pumped sample stream and, after sampling, to stow the cassettes in a sealed storage cylinder filled with preservative Fig. 5. We implemented a fluidics control system to convey sample water, preservative, sterilizer as needed, and to monitor differential pressure across the sample filters to detect clogging Fig. 6.

The prototype sampler is controlled by an embedded microcontroller, which detects the state of the sampler mechanisms and automatically moves the sampling module through a series of steps every time a sample is requested Fig. 6. The topside system controller was configured to interface through a cabled umbilical using through RS-232. This allows a user to trigger samples manually or configure the unit to take samples at regular time intervals. A drive motor, valves, and pressure transducers are contained in an oil-compensated housing Fig. 1. The microcontroller and motor control board are housed in an atmospheric pressure vessel, allowing deployment to 200 m depths (a next generation unit is being designed for 6000 m depth capability). Bench-top and pool tests were performed to prove the mechanical design and overall concept Fig. 2.

The cassette changing mechanism Fig. 4 proved robust and effective, consistently transferring sequential sets of filter cassettes and cover slides, which, when stacked in alternating sequence with the filter cassettes, were part of the strategy for isolating filters from each other Fig. 3. Upon receiving a programmed request from the user via the surface computer, the sampler performed a pre-flush operation Fig. 5.1, filling the entire fluid path with new sample water, and loaded a filter cassette across the aperture Fig. 5.2. Then sample water was pumped through the filter, and then preservative was drawn out around the newly sampled filter Fig. 5.3, before the sampler stowed the filter cassette in the take-up cassette storage. Next, a cover slide was loaded from the supply storage onto the stowed filter cassette, re-sealing and isolating the storage preservative bath contained within the sample storage cylinder. Finally, with the filter cassettes safely sealed away from the flow path,

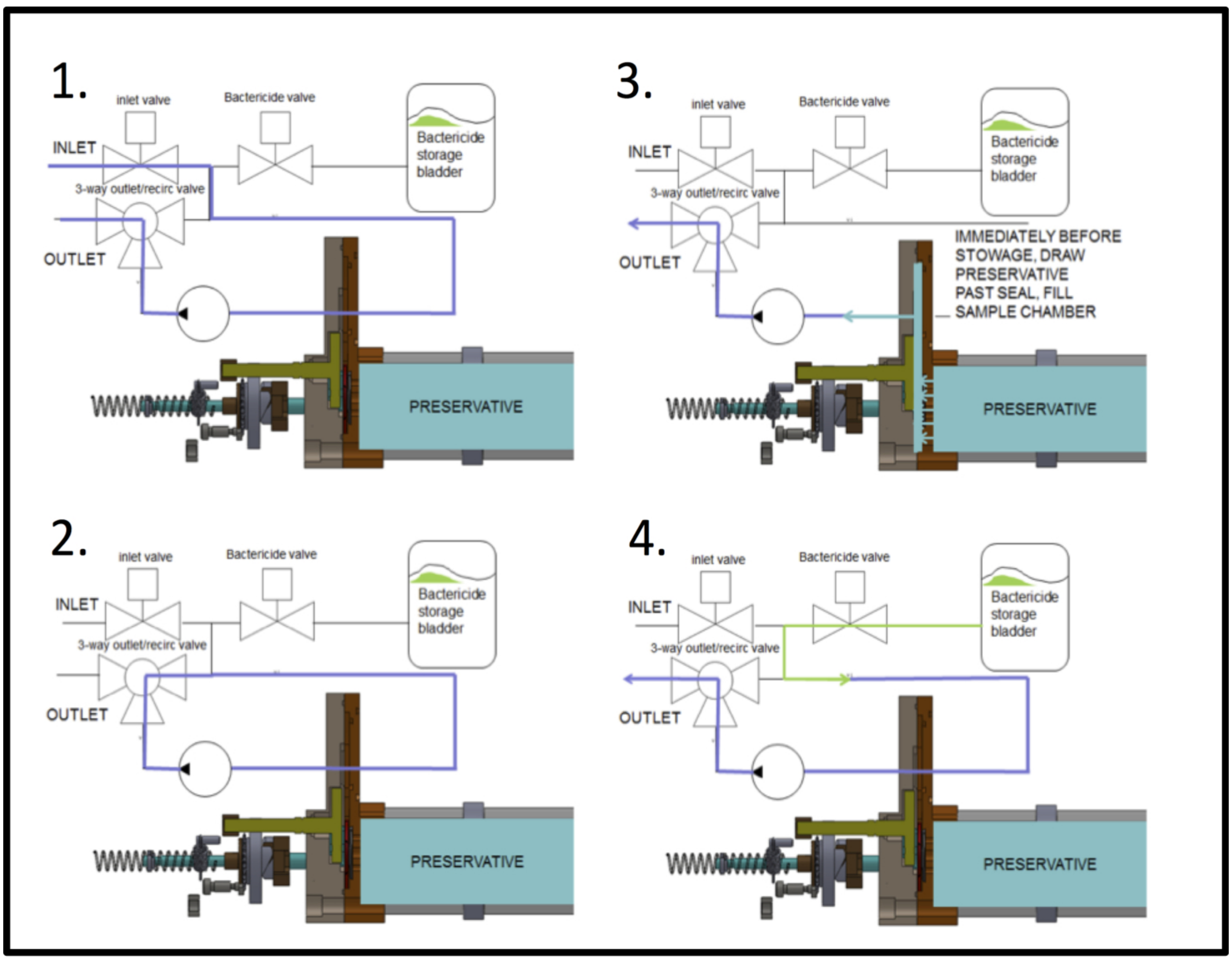


Fig. 5. Sequence of Sample Stages 1) inlet open to slurp water parcel 2) recirculation of water through filter stack 3) injection of preservative and 4) sterilizing system with biocide injection.

bactericide was successfully drawn into the aperture and flow path to disinfect in preparation for the next sample Fig. 5.4. This sequence was consistently repeatable**.** In addition, pressure across the cassette monitored by the controller showed a gradual increase until the maximum pump pressure was reached, indicating clearly that the sample stream was being directed through the filter without leakage.

Operational tests revealed a number of remaining challenges in the prototype design, particularly in the areas of mechanical design and ease of use. To reduce weight and cost, we designed the sample module to be driven by a single motor Fig. 2. This, coupled with the novel way we designed the three modules to stack together within a 230mm cylinder ended up requiring a complex drive system design, which included two rotating drives for slide transfer Fig. 4 and two linear functions for pushing slides to and from storage, all driven by a single motor. We found that the benefits of having only a single motor driving each module were outweighed by the cost of the complexity of the resulting mechanism. At every new test run or deployment, the slide storage cylinders must be removed from the sampler, and re-loaded into the sampler containing fresh (sterile) filter slides. In the prototype design, the slide storage cylinders sealed against the changer housing with circumferential O-ring glands Fig. 4, requiring axial insertion of the cylinder ends into female receptacles in the housing. Axial insertion resulted in significant disturbance to the system at every change-out – the front and rear housings Fig. 1 and Fig. 2 had to be spread apart from each other to allow removal of the cylinders. It also resulted in disturbance to the slides themselves, which would result in cross-contamination of actual samples. These operational difficulties and the need to control cross-contamination provide clear design modification challenges in the next phase goal of seeking to turn the MAB into a field deployable unit. At the heart of the MAB is the functionality developed through the prototype of being able to store, transfer, and process several hundred distinct samples.

IV. Conclusion

In this project, we developed a prototype sensor for low-cost, high sample capacity modular, autonomous biological sampling (MAB). The unit is designed for filtering and preserving 3 distinct biological size-classes (including bacteria), and is deployable in any aquatic setting from a variety of platform modalities (AUV, ROV, or mooring). A feature of the sampler design can be called “Question-driven adaptability” – the number of filters can be reduced to shorten the overall length (for AUV deployment, e.g.). Furthermore all three storage modules can be loaded with, for example, 150 µm

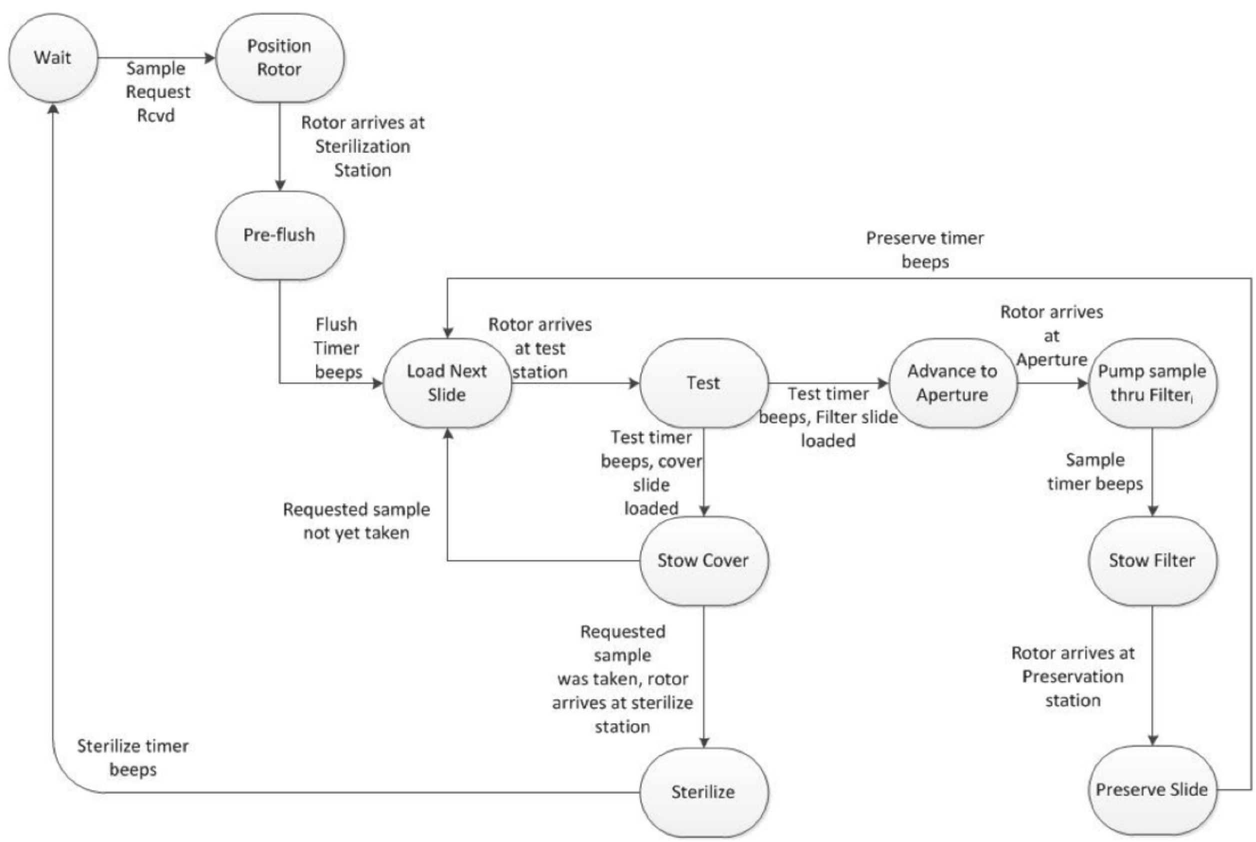


Fig. 6. MAB state machine sample sequence

mesh size filters only, for a scientist interested solely in zooplankton. In fact, if any filter medium can be loaded into the filter slides, they can be used in the sampler. This potentially broadens the scope of the sampler beyond fields of biology to include filtering for suspended sediment in the water column or geochemical tracers. The development of our prototype system has lead to a new and novel resource for researchers interested in enhanced biological sampling of the oceans.

We hope to build upon this prototype effort, eventually leading to a series of commercial sampler products that will be used in diverse marine settings. The design insights gained during the prototype project have informed our understanding of the biosampler, thus improving design plans for subsequent field units. The most valuable innovation we are carrying forward from the prototype system is the successful slide filter exchange mechanism.

The development of a low-cost high-capacity smart biosampler that has the ability to store samples for extended periods of time for later analysis will satisfy the critical need to characterize understudied areas of our oceans. The modular autonomous biosampler (MAB) system has the potential to dramatically increase our remote long-term biological surveillance capabilities.

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