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An Alternative Method to Niskin Sampling for Molecular Analysis of the Marine Environment

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Abstract: The development of low-cost, open-source Remotely Operated Vehicle (ROV) systems has provided almost unrestricted access for researchers looking to monitor the marine environment in ever greater resolution. Sampling microbial communities from the marine environment, however, still usually relies on Niskin-bottle sampling (ROV or Conductivity-Temperature-Depth sampler (CTD) based), a method which introduces an inaccuracy and variability that is incompatible with metatranscriptomic analysis, for example. Here, we describe a versatile, easily-replicated platform which achieves in situ mRNA preservation, via the addition of RNAlater to filtered microbial cells, to enhance ROV or CTD functionality.

Keywords: remotely operated vehicle; metatranscriptomics; Niskin
an ROV mounted Niskin system to study metatranscriptomic profiles, we were struck by the contrast between the antiquated nature of this traditional and inaccurate sampling technique, and the low cost, high-performance simplicity of the ROV system upon which was mounted. To this end, we looked to develop a versatile, easily replicated RNA sampling platform ("RNA Automated Preservation in situ Device, RAPID") inspired by low-cost, high-performance and simplicity. It is well established that in situ mRNA preservation can be achieved rapidly and simply through the addition of RNAlater (Ambion, Austin, TX, USA) to microbial cells (Ottesen et al., 2011) [8].

With this premise in mind, we looked to design a system that could both concentrate and preserve samples in a rapid, simple and low cost manner. Utilising off the shelf components we assembled and tested an Arduino (Leonardo) controlled dual pump system [9], capable of pushing seawater through a suitable filter unit, prior to the delivery of RNAlater (Figure 1). With motors and electronics encased and powered from 12 V supply (4 × AA batteries) within a permanently sealed waterproof junction box (Model: a16030800ux0347, Uxcell, Hong Kong, China) (Figure 2), and filtration units and RNAlater reservoir (saline drip bag (Model: GMEPN-UK-72813179, Amazlabs, Guandong, China)) external for easy replacement and retrieval, our system was mounted on an OpenROV (OpenROV, Berkeley, CA, USA) (rated to 100 m depth) for testing [10]. Pumps were mounted alongside each other and tubing joined via a T-junction with one-way values (Model: 1024989, Online Car Parts Ltd, Lancashire, UK) attached to the (external) Swinnex 25 mm filter assembly (Merck Millipore, Hertfordshire, UK). Initial trials with centrifugal pumps (adapted from a NERF Electrostorm water pistol; Hasbro, Pawtucket, RI, USA) revealed rapid degradation of internal components exposed to seawater and RNAlater, so we favoured a peristaltic pump option (Model: A518, ZJchao, Guandong, China). Any filter assembly (and filter type) capable of withstanding pressure can be used (we have utilised 25 mm and 47 mm filter assemblies, as well as the Sterivex system). The Arduino was mounted on the lid of the box, so that in the situation of structural integrity being lost, water damage to the circuit would be minimised (total immersion in silicon oil is another simple way to reduce pressure effects). Nevertheless, replacement of the junction box with a more robust structure may be necessary to go beyond 100 m depth. Whilst we developed here a single filter sample system, the addition of simple controlled distribution valves will provide the opportunity for numerous samples to be taken and preserved in procession. Following activation of pump 1, seawater is pushed through the filter assembly at a rate of ~2.5 mL/s (we achieved filtration of ~500 mL through a 0.22 µm Sterivex Filter in 4 min (Merck Millipore, Hertfordshire, UK)), applying different filters varies the rate of flow, as does biomass accumulation on the filter, until pump 2 is engaged for a 10 s flooding with ~27 mL of RNAlater. Although not instantaneous, the sample is not subjected to any temperature, pressure and/or light variation (unless the ROV is operated to specifically induce such conditions) and filtration/preservation is performed rapidly in situ. This potentially represents a significant improvement in both accuracy of transcript profiles and rapidity in comparison with current sampling procedures which usually rely on a delay for filtering on board ship following sample retrieval.

For samples where it is crucial to preserve the transcriptional profile immediately, pumps 1 and 2 can be run simultaneously to bring RNAlater into contact with the seawater immediately prior to filtration or bag collection. Following retrieval of the ROV to the surface and RNA extraction in the laboratory, no difference was observed in quality or quantity of total RNA obtained by Niskin or the on board system (Figure 3), thereby proving the principle that sampling via systems of this type can provide sufficient and suitable RNA, which is by virtue of its processing more representative of the natural environment from which it is taken. In addition to costing less than £50 to build and being small enough to mount on low-cost, entry-level ROV systems (which provide visualisation, easy maneuverability, and often accurate depth and temperature data, in real time and therefore with the opportunity for responsive action), such a system can also be utilised in conjunction with more established CTD instrumentation. In the spirit of the open source ethos, we invite others to join us and take up the challenge in testing and developing improved versions of this versatile system that has the potential to revolutionise the molecular analysis of the marine environment [11].
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**Figure 1.** Configuration of Nucleic Acid Preservation device. RNAlater is stored within a saline drip bag to minimise pressure effects. Proximal and distal one way valves serve to ensure filter remains immersed in RNAlater following preservation. Dashed line denotes components contained within pressure and water resistant shell.

**Figure 2.** Components within the casing and their configuration (Prototype 1, with NERF Electrostorm motors). L200 × W120 × D76 mm.
Figure 3. Total RNA (112 ng/µL; RIN 28S:18S score, 8.0) extracted from approximately 500 mL of natural seawater from Plymouth Sound preserved by RAPID sampling, analysed by 2100 Bioanalyzer (Agilent, Santa Clara, CA, USA).

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Conflicts of Interest: The authors declare no conflict of interest.

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