Technical Note

An alternative method to Niskin sampling for molecular analysis of the marine environment.

Jonathan Teague[1,2,3], Thomas B. Scott[2], Sanjay Sharma[1], George Graham[4] and Michael J. Allen[3,*]

[1] Plymouth University, Drake Circus, Plymouth PL4 8AA, UK
[2] Interface Analysis Center (IAC), HH Wills Physics Laboratory, Tyndall Ave, Bristol, BS8 1TL, UK
[3] Plymouth Marine Laboratory (PML), Prospect Place, Plymouth, PL1 3DH, UK
[4] Sir Alister Hardy Foundation for Ocean Science (SAHFOS), Citadel Hill, Plymouth, PL1 2PB, UK

* Correspondence: mija@pml.ac.uk; Tel.: +44-1752-633100

Academic Editor: Antonio Trincone
Received: 10th March 2017; Accepted: 31st May 2017; Published: TBD

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 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

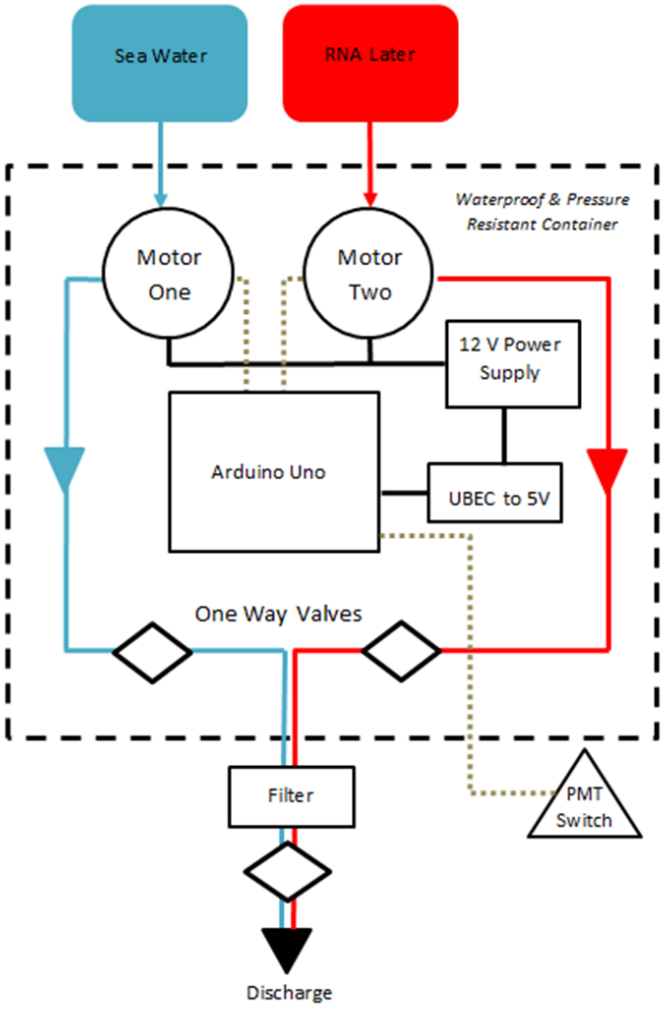
Based on the modified Nansen bottle (invented in 1894); the Niskin bottle (1967), invented just a few years after the discovery and characterisation of mRNA, was developed for the retrieval of seawater samples to the surface (Hill, 1900; Niskin, 1966; Cobb, 1990) [1-3]. Traditional Niskin sampling still dominates oceanic analysis, while metatranscriptomic (whole community mRNA profiling) based techniques have revolutionised our understanding of the function of mixed community assemblages at the molecular level (Gilbert et al, 2011) [4]. Together, they have provided a much needed insight on the fundamental workings of global biogeochemical cycling. However, while metatranscriptomics suffers from a necessity to reduce technical variation as much as possible to allow meaningful interpretation of results, it is stifled by the inaccuracy and variability that is irrevocably associated with current Niskin-based sampling methods. Whilst cellular mRNA profiles can respond to environmental insults within milliseconds, the mandatory transcriptional alterations induced by Niskin sampling, which subjects samples to unavoidable exposure to differences in pressure, temperature and light, in addition to the inherent temporal delay, is difficult to circumvent. This irreconcilable observation has stimulated the development of many *in situ* profiling technologies for the marine environment (Feike et al, 2012; Taylor et al, 2015; McQuillan and Robidart, 2017) [5-7], however these solutions have not gained dominance or widespread use as yet, primarily due to cost restrictions.

In tandem to the dawning realisation that the majority of current marine transcriptomic and metatranscriptomic analyses are inherently inaccurate, the development of low cost open source ROV systems has provided easy access (to the top 100 metres of the ocean at the very least) for researchers looking to monitor, and sample, the marine environment in ever greater resolution. Whilst utilising

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 is was mounted. To this end, we looked to develop a versatile, easily replicated RNA sampling platform ("**R**NA **A**utomated **P**reservation ***i****n situ* **D**evice, 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 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 12V supply (4 × AA batteries) within a permanently sealed waterproof junction box (Model: a16030800ux0347, SourcingMap) (Figure 2), and filtration units and RNAlater reservoir (saline drip bag [Model: GMEPN-UK-72813179, Amazlabs]) external for easy replacement and retrieval, our system was mounted on an OpenROV (rated to 100m depth) for testing. Pumps were mounted alongside each other and tubing joined via a T-junction with one-way values (Model: 1024989, Carparts online) attached to the (external) Millipore Swinnex 25 mm filter assembly. Initial trials with centrifugal pumps (adapted from a NERF Electrostorm water pistol) revealed rapid degradation of internal components exposed to seawater and RNAlater, so we favoured a peristaltic pump option (Model: A518, ZJchao). 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 though 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 minutes), 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].



99

100 **Figure 1.** Configuration of Nucleic Acid Preservation device. RNALater is stored within a saline drip
101 bag to minimise pressure effects. Proximal and distal one way valves serve to ensure filter remains
102 immersed in RNAlater following preservation. Dashed line denotes components contained within
103 pressure and water resistant shell.

104

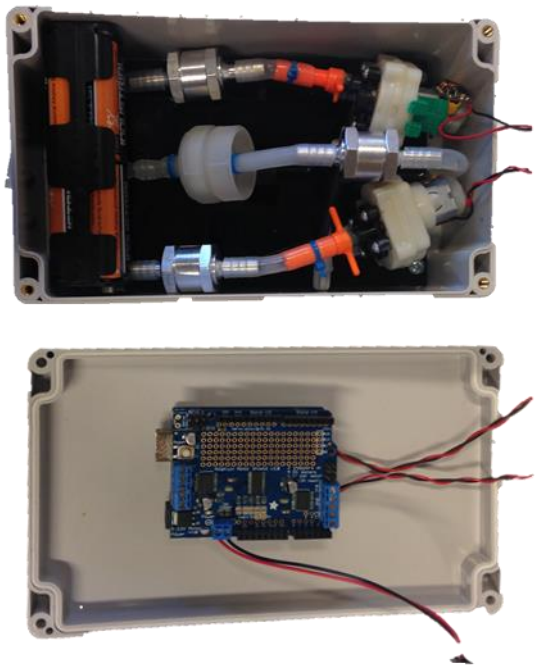


Figure 2. Components within the casing and their configuration (Prototype 1, with Electrostorm motors). L200 × W120 × D76 mm

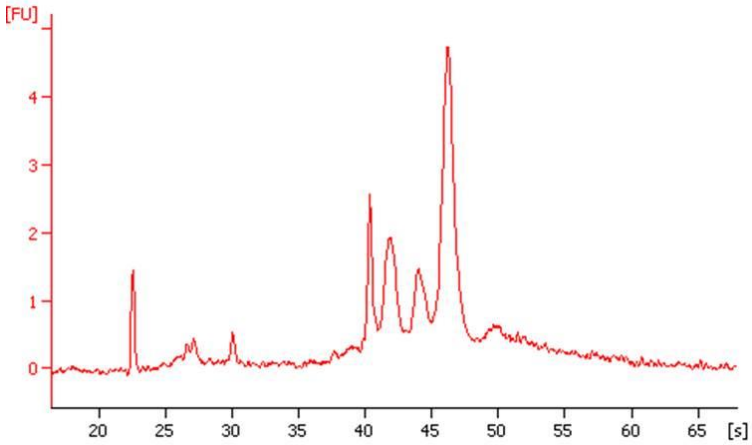


Figure 3. RNA (112 ng/µl; RIN 28S:18S score, 8.0) extracted from approximately 500 ml of natural seawater from Plymouth Sound preserved by RAPID sampling, analysed by Agilent Bioanalyser.

Acknowledgments: We would like to acknowledge the internally funded PML Research Program (awarded to M.J.A) and Cabot Institute Innovation Fund (awarded to T.S. and M.J.A.) for providing the funding for this study.

Author Contributions: M.J.A and J.T. conceived and designed the experiments; J.T. performed the experiments; M.J.A, J.T., S.S., G.G. and T.S. analyzed the data; M.J.A, J.T., G.G., S.S. and T.S. contributed reagents/materials/analysis tools; M.J.A and J.T. wrote the paper.

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

References

1. Niskin S.J.; Water sampler device. *US Patent Application US3489012A* **1967.**
2. Mill H.R.; The Pettersson-Nansen Insulating Water-Bottle. *The Geographical Journal* **1900** *Vol. 16*, No. 4, pp. 469-471.
3. Cobb M.; Who discovered messenger RNA? *Current Biology* **2015** *Vol. 25*, Issue 13, 29 June, pp. R526-R532, ISSN 0960-9822.
4. Gilbert, J.A.; Field, D.; Huang, Y.; Edwards, R.; Li, W.; Gilna, P.; Joint, I. Detection of large numbers of novel sequences in the metatranscriptomes of complex marine microbial communities. *PloS one* **2008**, 3 (8) p.e3042.
5. Feike, J.; Jürgens, K.; Hollibaugh, J.T.; Krüger, S.; Jost, G.; Labrenz, M. Measuring unbiased metatranscriptomics in suboxic waters of the central Baltic Sea using a new in situ fixation system. *The ISME journal* **2012**, 6 (2), pp.461-470.
6. McQuillan J.S.; Robidart J.C. Molecular-biological sensing in aquatic environments: recent developments and emerging capabilities. *Current Opinion in Biotechnology* **2017** 45 pp. 43-50.
7. Taylor, C.D., Edgcomb, V.P., Doherty, K.W., Engstrom, I., Shanahan, T., Pachiadaki, M.G., Molyneaux, S.J.; Honjo, S. Fixation filter, device for the rapid in situ preservation of particulate samples. *Deep Sea Research Part I: Oceanographic Research Papers* **2015**, 96, pp. 69-79.
8. Ottesen, E.A.; Marin, R.; Preston, C.M.; Young, C.R.; Ryan, J.P.; Scholin, C.A.; DeLong, E.F. Metatranscriptomic analysis of autonomously collected and preserved marine bacterioplankton. *The ISME journal* **2011**, 5(12), pp. 1881-1895.
9. https://www.arduino.cc/
10. https://www.openrov.com/
11. https://github.com/Teaguey08/RNA-preservation-Device/tree/Files