

A Miniaturized and Automated eDNA Sampler: Application to a Marine Environment

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Abstract—Environmental DNA (eDNA) analysis offers a time- and cost-efficient method to perform temporal and spatial observations of aquatic environments, providing a wealth of biodiversity data. Unfortunately, most protocols require manual collection of eDNA samples using highly skilled individuals to capture, filter and store the samples before they can be sent to a lab for analysis. Automated samplers exist to reduce this front-end collection burden, but they tend to be bulky and costly. Here we describe an eDNA sampler designed to be user-friendly and to automate the entire sample collection process. We describe and characterize our eDNA sampler, which offers multi-sample capture, preservation, and self cleaning to reduce cross contamination. DNA sequencing was performed on samples collected using the eDNA sampler as well as a traditional niskin bottle-based protocol at six stations in Bedford Basin, Nova Scotia, Canada. The two approaches showed similar algal and bacterial taxonomic compositions, demonstrating the ability of the eDNA sampler to generate results comparable to those obtained through gold-standard protocols.

Index Terms—eDNA, sampler, genomics, environmental sensors, biodiversity

I. INTRODUCTION

Increased human activity in marine environments has led to concerns over subsequent anthropogenic effects on aquatic ecology and environments. These include, but are not limited to: hypoxia, ocean acidification (OA), and elevated nutrients [1], all of which have been shown to negatively affect aquatic

ecosystems. Ocean acidification, caused by climate change, is of particular interest due to its effect on calcifying marine species such as coccolithophores as it can lead to malformation or dissolution of their skeletons [2]. Meanwhile, anthropogenic nutrient addition from industries such as aquaculture can affect aquatic community structure and promotes growth of organisms such as harmful algae to bloom (HABs). Algal blooms can affect aquaculture farms directly by harming fish via gill damage or causing toxigenic effects [3], as well as indirectly if a bloom renders a farm unsafe for human presence or consumption, leading to temporary closures [4].

With the previously mentioned effects, there has also been an increase in the need for dedicated monitoring programs. Previously, biological monitoring programs were limited to time-consuming and expensive methods such as taxonomic identification, however with the increase in nucleic acid sequencing databases and decreasing cost of sequencing, studying environmental DNA is becoming a viable option for biological monitoring [5]. Environmental DNA, or eDNA, refers to all DNA present in the environment, whether it be from organisms currently in the area, or DNA that sloughed off organisms passing through the environment [6], [7]. In the last decade there has been growing interest in the use of eDNA as a tool for targeted species detection and biodiversity assessments, and as a non-invasive technique expected to influence environmental management [8]. Aside from it being non-invasive, studying eDNA also allows for exploration into the changing microbial communities. This is advantageous as smaller organisms such as bacteria, archaea, and microeukaryotes are more closely related to biogeochemical cycles and have shorter generation times, making them particularly re-

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sponsive to environmental disturbance [9]–[13].

Therefore, there is currently a push towards environmental genomics for biomonitoring in areas such as aquaculture, fisheries, transportation, power generation (hydro, wind), pollution regulation, and oil and gas offshore infrastructure sites [10]. Studies have already shown that high throughput sequencing (HTS)-based metabarcoding of Foraminifera can be used as a rapid and cost-effective environmental assessment at fish farms [14], and that monitoring and preserving healthy microbiomes can increase sponge resistance to OA [2]. As well, quantitative Polymerase Chain Reaction (qPCR) analysis of eDNA has been investigated as a technique for identifying and quantifying HABs [15]. Aside from biomonitoring purposes, eDNA can also be used for early detection and temporal and spatial monitoring of non-indigenous species and is particularly useful for detecting cryptic invasive species [16], [17].

Traditionally, either filtration or precipitation are the two most used methods to capture eDNA from aquatic environments; however, because filtration tends to recover more DNA it has been the most used method [8]. Filtration methods are often labour intensive involving manual collection of samples using equipment such as niskin bottles, and ship time for offshore sampling. As well, these samples require separate pumping systems to filter water after it has been collected, and a method of preservation, often via freezing at $-80\text{ }^{\circ}\text{C}$. Therefore, in recent years there are increasing publications focused on creating more automated sampling equipment. These samplers range from single-filter systems (e.g. Smith-Root eDNA sampler, the C.L.A.M, and SASE), toward more complex multiple filter systems (McLane’s Phytoplankton Sampler and MBARI’s Environmental Sample Processor). Many samplers are not commercially available and each sampler is applicable for different scenarios based on the deployment duration, maximum depth rating, and chemicals/preservatives used. A brief list of current eDNA samplers is outlined in Table I.

Focusing solely on *in situ* instruments, MBARI developed the Environmental Sample Processor (ESP) Gen 1 and Gen 2 from 2001 to 2009 [19]. These were comprehensive labs-under-the-sea that performed sample collection and DNA extraction to feed PCR microfluidic devices, hybridization arrays, and sandwich assays. However, the ESP instrument costs several hundred thousand dollars and is highly complex to deploy and service, with final contracts typically in the millions of dollars. The latest generation of instrumentation from MBARI removes the integrated analysis, aiming to perform sample collection with preservation on underwater vehicles, followed by land-based laboratory genomics analysis [27]. The sampling capabilities of the ESP Gen 3 have an impressive 60 filter cartridges; however these cartridges have four screws per filter for a total of 240 screws, making it impractical for commercial use by aquaculture operators, deployment in city harbours, or the like.

Streamlined samplers aim to improve scalability for observing eDNA *in situ*, including: the Subsurface Automated Sampler for eDNA (SASE), PolyWAG (Water Acquired Genomics), and the CLAM (Continuous Low-Level Aquatic

Monitoring). These systems are priced in the thousands of dollars range and are targeting citizen science by making eDNA sampling more accessible. Most have a single filter, tend not to carry preservation or cleaning reagents, and are suitable for short-term (hourly, daily) deployments. While some have longer-term deployment capability (SaSe unit has onboard preservation capability), many lack the ability to self-clean with acids or bleach, nor do they flush the sample inlet/intake to minimize biofouling and cross-contamination. The polyWAG system can be categorized at the same end of the spectrum as the SaSe unit. However, unlike the other low-cost samplers, it offers 24 filters with self-cleaning and self-preservation (ethanol) being integrated. Unfortunately, cleaning is achieved by air flushes. The added automation increases cost to \$3,000 - \$5,000. Furthermore, these designs seldom consider form factors that are amenable toward integration with platforms or autonomous vehicle payload constraints.

Here we present a novel autonomous eDNA sampler that uses an innovative design to collect, filter, and preserve a water sample all within single compact instrument, shown in figure 1. The sampler is intended to be easy to use in field operations minimizing operator involvement in the process, and thus reducing the time to change filters and service the instrument. The fully assembled sampler can collect up to 9 discrete samples per deployment, which can be immediately preserved using RNeasy[®] (Ambion, Inc.). Unlike other samplers, the 9-filter cassette (figure 1b) is easily changeable on site in under 5 minutes to allow for immediate redeployment of the instrument and analysis of the filled cassette either in the field or back at the lab. Furthermore, the unit is self-cleaning (with acid) and all tubing is internal to the instrument housing (not exposed), preventing catastrophic snags that decouple fluid lines during deployments. The compact design is single person carry-able with dual handles that make transport and deployment simple and easy. Finally, we have included an external trigger line to enable optical, acoustic, vision, or positional synchronization and collection of eDNA samples. We anticipate the user-focused design of our sampler will lead to adoption across a wide range of sectors as it greatly standardizes and simplifies the collection of eDNA samples, thereby improving reliability and repeatability for the eDNA front-end portion of the analysis pipeline.

II. METHOD AND DESIGN

A. Chemicals

The Dartmouth Ocean Technologies Inc. (DOT) eDNA sampler utilizes three chemicals: RNeasy, 5% HCl, and Milli-Q ultrapurified water. RNeasy is used to preserve collected samples, 5% HCl is used to clean the system fluid lines and backflow the sample inlet, and Milli-Q is used to flush the system between protocol steps. The 5% HCl and Milli-Q are effective at reducing cross-contamination that might take place in the system tubing and manifolds between sampling events. Unless otherwise stated, chemicals used in this study were of analytical grade and supplied by Fisher Chemical (Waltham, MA, USA).

TABLE I
EDNA SAMPLERS IN THE LITERATURE AND ONLINE

Year	Instrument	Organization	Depth	Filter Numbers	Filter	Preservation	Self Cleaning
2001	Environmental Sample Processor (ESP) Gen 1 [18]–[20]	Monterey Bay Aquarium Research Institute (MBARI)	50m	100	25 mm Filter	Y	Y
2009	Environmental Sample Processor (ESP) Gen 2 [20]–[23]	Monterey Bay Aquarium Research Institute (MBARI)	50m ¹	100	25 mm Filter	Y	Y
2012	Modular Autonomous Biosampler (MAB) [24]	Cellula Robotics Ltd.	200 m	200	47 mm Filter	Y	Y
2014	Continuous Low-Level Aquatic Monitoring (C.L.A.M) [25], [26]	Aqualytical	6.1 m	1	47 mm SPE Disk	N	N
2015	Environmental Sample Processor (ESP) Gen 3 [21], [27]	Monterey Bay Aquarium Research Institute (MBARI)	300 m	60	25 mm Durapore Filter 0.22µm	Y	Y
2018	eDNA Sampler [28]	Smith-Root	Surface	1	47 mm Filter	Y ²	N ³
2019	<i>in situ</i> Autonomous Biosampler (IS-ABS) [29]	CIMAR	150 m	16	Sterivex Filter 0.2 µm	Y	Y ⁴
2020	PolyWAG (Water Acquired Genomics) [30]	Oregon State University	-	24	47 mm Filter Disc	Y	Y
2021	Subsurface Automated Sampler for eDNA (SASe) [31]	National Oceanic and Atmospheric Administration (NOAA)	55 m	1	Sterivex Filter 0.22 µm	Y	N
2022	Large Volume eDNA Sampler [32]	Woods Hole Oceanographic Institution (WHOI)	6000 m	12	PES filter 0.2 µm	N	N
2022	eDNA Sampler	Dartmouth Ocean Technologies Inc. (DOT)	20 m	9	25 mm Filter	Y	Y

¹ Depth can be increased to 1000 m by attaching an additional component to depressurize water before processing.

² Requires self-preserving filter

³ Sterilized Filter is provided but lacks acid cleaning protocol

⁴ Cleaning is performed with *in situ* water.

B. System Overview

The DOT eDNA sampler features a simple modular approach that has three detachable sections: filter cartridge, electronics section, and fluid storage section, see figure 1. The fully assembled unit is 72.1 cm × 16.8 cm and weighs 11.3 kg in air and 3.3 kg in salt water. It is capable of cleaning between sample captures, preservation of captured samples and has 9 discrete filters, 25 mm in diameter. Different filter membranes can be loaded into the filter holders (Advantec 43303010, Polypropylene), thus allowing for a wide variety of membrane materials and pore sizes to be used based on targeted species. The eDNA sampler’s filter cartridge is made from Polyetheretherketone (PEEK) material and holds the 9 filter holders. Once the filter cartridge is loaded with clean filters it can be attached to the electronics section of the eDNA sampler. This fast swap approach allows for multiple filter cartridges to be prepared and then loaded into the sampler as needed. The filter cartridge is secured by 3 knobs that are keyed to the electronic section to avoid assembly error.

The eDNA sampler’s electronics section is the core of the instrument. It houses a pump and custom valve tree, along with a custom printed circuit board (PCB) for automation and data logging. The PCB and software will be described in the Architecture section below. The valve tree consists of the fluid routing manifold, pressure sensors, tubing interconnections, and solenoid valves for the sampler. The valve tree also has ports that are used to fluidically couple to the filters on the filter cartridge, and to access the fluid bags loaded with reagents and stored in the fluid section of the sampler.

The eDNA sampler’s fluid storage section houses and protects all the required fluids and the “PIXIE”, a novel 4-channel fluorometer. The fluids stored in this section are as follows: 5% HCl (cleaning), RNALater (preservation), purified Milli-Q water (rinsing) and waste. The fluids are stored in 100 and 500 mL Labtainer™ BioProcess Containers (BPC) and connected to the Electronics section with 1/4 - 28 ports. The waste bag is

used to hold chemicals that are deemed not safe to flush into the ocean or surrounding waters. The four channels of the PIXIE fluorometer are configured with optical filters and LEDs for the detection of algal indicators chlorophyll, phycocyanin, phycoerythrin and turbidity by default. However, crude oil and rhodamine have also been tested using this fluorometer. The fluorometer can also be used in a standalone configuration and measures 58 mm × 122 mm, and weighs 370 g. Future manuscripts will present correlations observed between eDNA samples and fluorescence measurements collected simultaneously with the onboard PIXIE.

C. Automation Protocol

Figure 2 illustrates the simplified fluid schematic of the eDNA sampler. Two configurations are shown; a push configuration in figure 2(a) and a pull configuration in figure 2(b). These two configurations describe how the pump is used to deliver sample water to the filter membranes. In the push configuration, the syringe pump draws in sample from the environment and then pushes the fluid sample across the membrane. Therefore, the sample fluid is initially in contact with the pump innards before filtration and thus have much higher dead volumes (3 mL) that need to be cleaned between sampling events. By contrast, in the pull configuration, the syringe pump draws in sample through the filter membrane and only the filtered water (permeate) is in contact with the syringe pump/barrel. Therefore, the sample fluid is not in contact with the pump before filtration and the dead volumes are considerably lower (<1 mL). Minor modifications to the tubing connections in the fluidic section of the system permit the sampler to be run in either configuration. The push mode is more appropriate at low ambient pressures to avoid bubble formation during pumping, but it requires more flushing to clean the syringe pump. The pull configuration is generally preferable as it minimizes sample cross-talk/carryover in the pump and is pragmatic at depths greater than 10 m (1 bar) when bubble nucleation becomes less problematic.

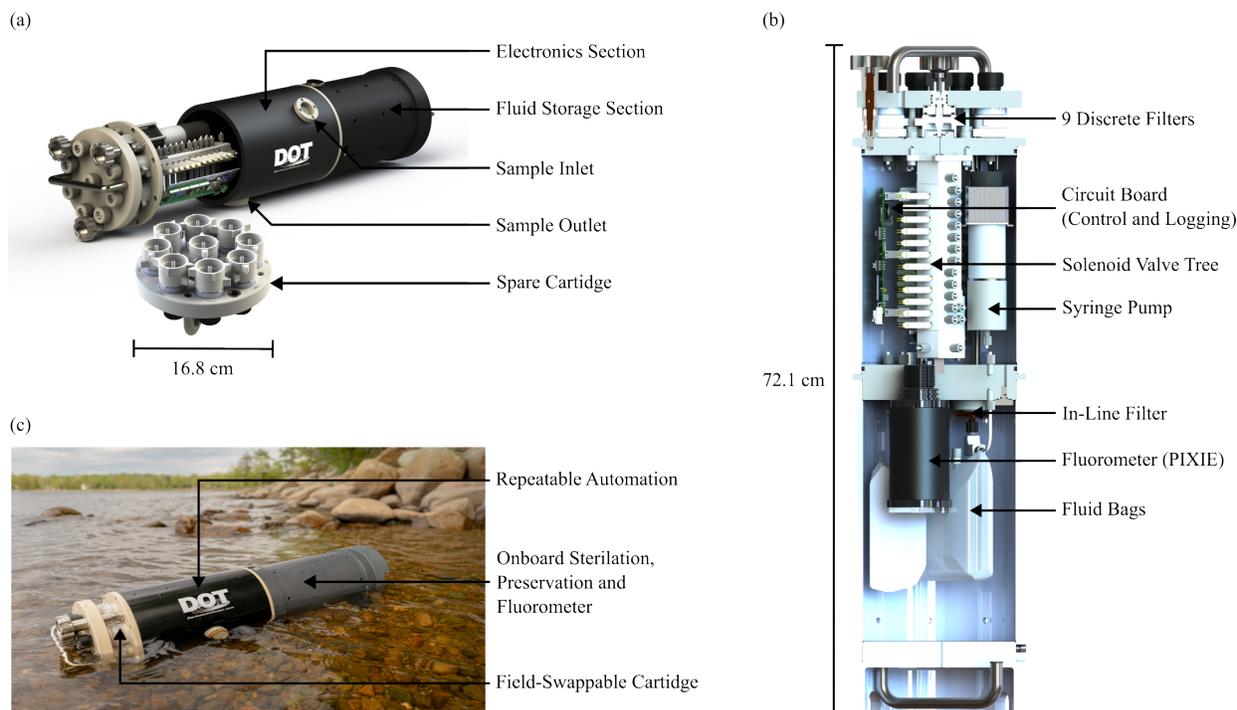


Fig. 1. (a) 3D CAD rendering of the DOT eDNA sampler with partially exposed electronics section. (b) Cross-sectional view of the DOT eDNA sampler, highlighting all compartments and fluid storage bags. (c) Fully built DOT eDNA sampler deployed in Grand Lake, Nova Scotia, Canada.

In either configuration, the system initially draws in fluid across a 35 μm PEEK mesh positioned at the sample inlet. While 35 μm was selected to protect the internal components of the system, larger mesh sizes, 100 to 300 μm , are possible and they are being evaluated with results to be presented at a later date. The system automation is controlled by custom scripts that coordinate valve operation and flow rates, whilst logging pressure and fluorescence data. Figure 2(c) shows the high-level sampling protocol. Upon receiving a “sampling” signal (fluorescence threshold, or scheduler based, or external trigger activation), the system enters a cleaning mode; labelled “Acid Clean”. This involves pulling 9 mL HCL from the bag and its corresponding solenoid valve across the the acid bypass line. The unit then pushes the 9 mL HCL out the sample inlet port to clean the intake. The unit again pulls 9 mL HCL and pushes that to the sample outlet to completely fill the internal lines with 5% HCL. To remove the 5% HCL, Milli-Q flushing is used, where 9 mL of Milli-Q is pushed across the sample inlet and another 9 mL of Milli-Q is pushed to the sample outlet. This step is called the “MQ Flush”. The unit then proceeds to a “Sample Prime” step. This step pulls 9 mL from the sample inlet and flushes the lines, similar to the MQ flush step. All steps in the Acid Clean, MQ Flush, and Sample Priming use a flow rate of 20 mL/min in aspirate and dispense operations, and volumes of fluid used by the system during these preparatory steps are: 18 mL 5% HCL, 18 mL Milli-Q, and 18 mL of sample. The unit is now ready to capture sample on the target membrane.

The selected membrane (M1 through M9) is then fluidically

coupled into the flow stream to capture sample by opening the respective valves. A user-specified flow rate can be used during sample collection, but typically this ranges from 5 to 60 mL/min. In this work, samples were captured at 60 mL/min. For example, an often used 125 mL sample collection volume required 15 minutes at 20 mL/min; 6 minutes of sample collection (dispensing in push configuration), 6 minutes of emptying the syringe pump to waste (effectively a 50% duty cycle), and 3 minutes of pause time to allow pressure equalization in the fluid lines. While the “Sample Capture” step is being run, the “Thresholds Flow Diagram” is being constantly monitored to ensure the pressure limits, volume limits, and time limits are not being exceeded. These limits are user-specified by use case, and were 40 psi, 125 mL, and 30 minutes in this study. The thresholds algorithm runs concurrently with the pumping and ensures a stable pressure plateau is reached before changing the valves to prevent surging and uncontrolled fluid routing. This also prevents dead-end pressure buildup and damage caused to the system. The threshold flow first checks pressure limits, and if excess pressures are detected, the flow rate is decremented by 40% to a lower limit, hereafter referred to as a “pressure plateau”. By slowing the flow rate, more volume can be delivered (and thus more DNA mass) to the membrane without exceeding the maximum trans-membrane pressure. Ultimately, the loop is exited when either the time or the volume limit is reached and the sampling protocol resumes. Once the “Sample Capture” phase is complete, the system dispenses 6 mL of RNAlater across the membrane to preserve the DNA. If another sample is scheduled, the process in figure

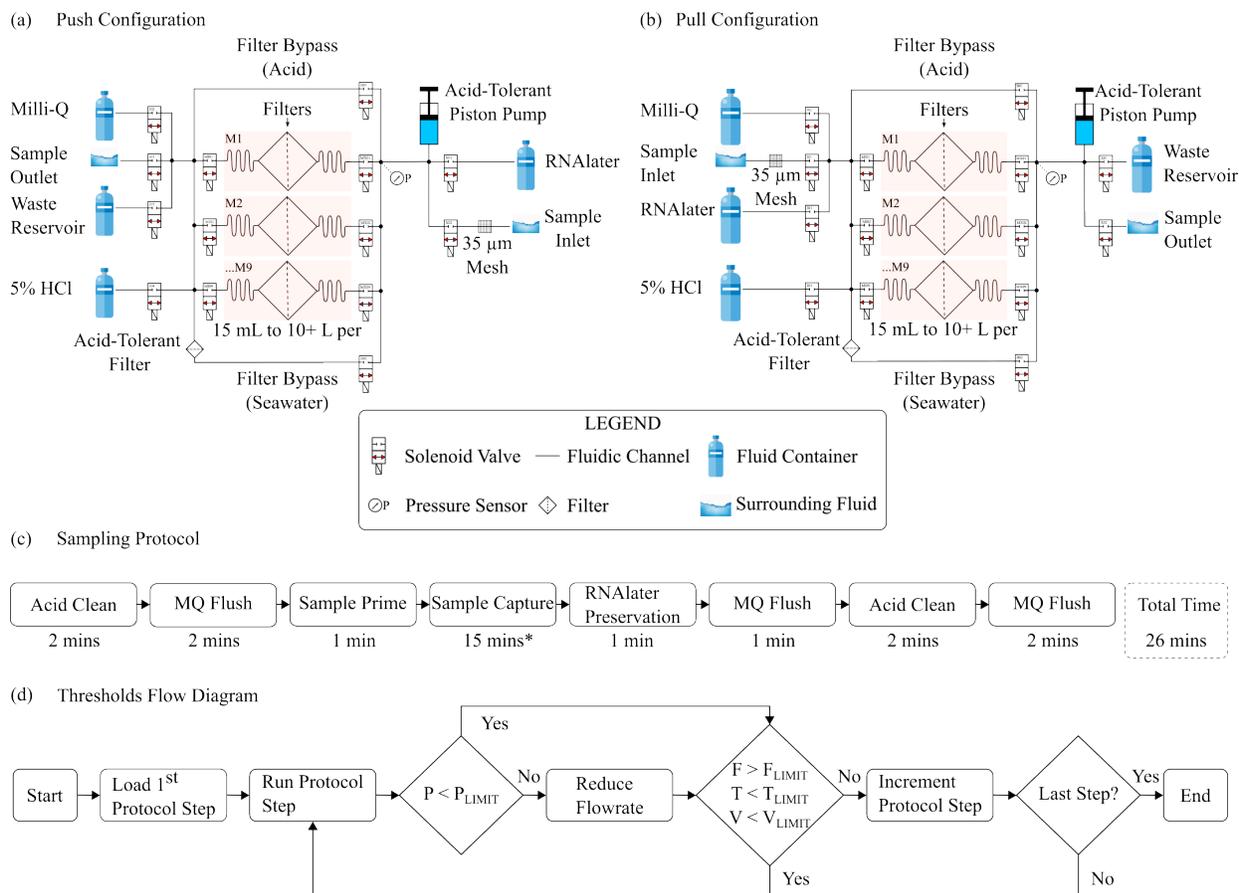


Fig. 2. (a) Fluid Schematic for the DOT eDNA sampler shown in a Push Configuration. (b) Fluid Schematic for the DOT eDNA sampler shown in a Pull Configuration. (c) Protocol used to capture and preserve sample then clean system. (d) Thresholds flow diagram used to load and run protocols within a safe user-specified operating region (F - Flowrate, P - Pressure, T - Time, V - Volume). * The sampling time is dependent on protocol-specified flowrate and fluid turbidity. The time shown above is for 20 ml/min in ideal conditions.

2(c) repeats; otherwise, the sampler enters into a low power state waiting for the next trigger.

D. System Architecture

The eDNA sampler's protocol is accomplished with the system architecture shown in figure 3. Due to the varied electrical requirements of the sub-components, the system has regulators for generating multiple voltages ranging from 3.3 to 12 VDC, all sourced by a battery or power supply input of 7 to 24 VDC. The wide range of voltage input allows for flexibility in the platforms used for deployment (UAV/USVs, Buoys, Moorings, etc.). The system is controlled using an ARM Cortex M4 microcontroller (STM32F411) running at 84 MHz and configured as a Real Time System (RTS) that is governed by several interrupts and low level controls to ensure precise timing. The single microcontroller manages the syringe pump, data logging, communication, and protocol execution. The syringe pump (a custom variant of the LPDA1750330H, Lee Company Ltd.) is powered with a stepper-motor driver circuit (DRV8834, Texas Instruments) along with an optical quadrature encoder to aid the precise tracking of the volume used. The 26 solenoid valves used by the system are driven by

a spike-and-hold circuit (DRV8860, Texas Instruments) that allows powering 32 valves without excessive current load. The DRV8860 is a serial connectable device and allows for a modular design for the expansion of solenoids that can be used by the system. The eDNA sampler makes use of a 16-bit ADC (ADS1115, Texas Instruments) module that is able to read the pressure sensor in a wheatstone bridge configuration with its builtin programmable gain amplifier (PGA). The amplified signal permits differential trans-membrane pressure measurements to be read for ensuring the membranes are used within manufacture specifications (typically under 4 bar, 60 psi). An additional pressure sensor is used to read the ambient pressure of the environment and the depth of the sampler. The sampler stores all data on an internal 32 GB microSD card with timestamped files and folders. Users interface with the eDNA sampler through either Bluetooth (via a smartphone application) and/or through RS-232 and a personal computer terminal. These both permit operational commands to be sent to the sampler and are also conduits for transferring data to/from the system; for example, setting scheduled sampling times via the real time clock (RTC) and/or for retrieving pressure and fluorometer data per membrane/sample. At Idle

the system draws 1 W, while sampling it draws 6.5 W.

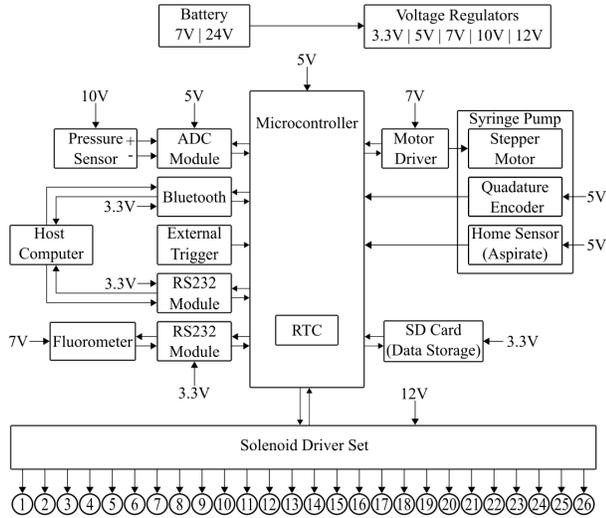


Fig. 3. Architecture diagram for the DOT eDNA sampler showing internal electrical connections and components along with interfaces to the external world.

E. DNA Extraction and Sequencing

DNA from all samples was extracted using the Qiagen DNeasy plant mini kit using a protocol based on Zorz *et al.* 2019 [33] with minor modifications of incubating samples at 52 °C for 1 hour and eluting the DNA by running the same 50 μ l of Qiagen AE elution buffer (AE buffer) for genomic DNA in order to maximize DNA concentration. After extraction, DNA concentration of each sample was measured using a NanoDrop™. 10 μ l of DNA extract was sent to the Integrated Microbiome Resource lab (IMR) at Dalhousie University for Illumina amplicon sequencing of the V4-V5 variable region of the 16S ribosomal RNA gene using the following primers: 515F = 5'-GTGYCAGCMGCCGCGTAA-3', 926R = 5'-CCGYCAATTYMTTTRAGTTT-3' [34], [35].

F. Bioinformatics

Raw sequencing data was processed through the QIIME2 2019.7 pipeline [36] as outlined by the Microbiome Helper developed by IMR [37]. Reads underwent denoising using Deblur (QIIME2 plugin version 2019.7) [38], after which Deblur distinguishes sequence variants differing by as little as one nucleotide and labels them as amplicon sequence variants (ASVs) [39]. After ASVs were assigned, they were assigned to taxonomic groups using the SILVA 132 database [40], [41]. Chloroplast sequences were further classified using the PhytoREF database [42]. Phyloseq [43] was used to make all plots using R Studio [44] and the packages ggplot2 [45], and ggpmisc [46].

III. RESULTS

A. Pressure Analysis on Bench

Before *in situ* testing, the unit was characterized on the bench with bottled seawater from the Bedford Basin, Nova

Scotia that was collected on May 20, 2022. Figure 4(a) illustrates the pressure data from an initial bench test run of the eDNA sampler and highlights a typical pressure profile. A volume of 500 mL of sample was filtered across a 0.2 μ m filter, with an aspirate flowrate of 10 mL/min and a dispense flowrate of 20 mL/min, yielding an average effective flow rate of 6.67 mL/min. When deployed *in situ*, the rates described in the Method and Design section were used, but for bench testing slower flow rates were used to permit debugging.

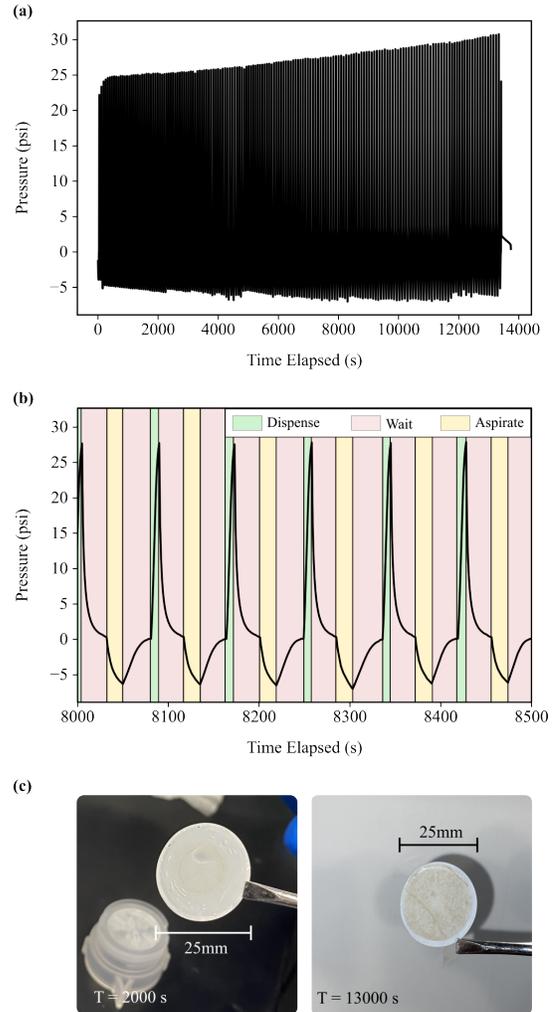


Fig. 4. (a) Pressure data captured during the sampling process on a 0.22 μ m polycarbonate filter membrane. (b) Same data as in (a), but zoomed-in pressure cycle to highlight the syringe pump control scheme used. (c) Retentate on the porous membrane, during the early stages of filtration and near the end of filtration. The filter is removed for DNA extraction and downstream processing and analysis.

After 13,000 seconds, the user-specified pressure limit of 40 psi was not reached and thus the automated protocol stopped capturing sample when the volume limit was reached. Figure 4(b) is an zoomed-in portion of figure 4(a) from 8,000 to 8,500 seconds to highlight the pumping-phases/cycles. Figure 4(b) depicts the pump cycle repeated; an aspirate step for 18 seconds (sample inlet to pump, negative pressure buildup), followed by a wait step for 27 seconds (pressure leveling off

to zero), followed by a dispense step for 9 seconds (pump to membrane, positive pressure buildup), and a final wait step of 27 seconds (pressure leveling off to zero). The syringe pump was active for 75 minutes; however, due to the extra wait time (67% of the cycle), the total filtration time required was 228 minutes. The added wait time was performed only during bench testing of the system, for refining and optimizing the protocol. Figure 4(c) shows minimal biomass accumulation on the filter at 2,000 seconds near the start of a filtration run, and substantive biomass accumulation on the filter at 13,000 seconds near the end of a filtration run. Following successful verification of the automated protocol on the bench, the unit was ready for field trial validation studies.

B. Field Trial in Bedford Basin

After multiple successful test runs off of the jetty at the Centre for Ocean Ventures and Entrepreneurship (Dartmouth, NS), the eDNA sampler was brought into the field and deployed off of a vessel during a transect of the Halifax Harbour. Of the 7 stations where the sampler was deployed, 6 resulted in successful sample capture (figure 5), with Station M4 not fully captured because the user-specified pressure plateau was not set by the user prior to deploying the sampler (data not shown). At all other stations, the user-specified pressure plateau was set to 5.8 psi and the eDNA sampler was programmed to take a single 125 mL sample after a 1-minute delay to allow time to deploy down to 5 m depth. Water was filtered through 25 mm diameter, 0.22 μm polycarbonate (PC) membranes (Millipore). The sampler was then pulled back up onto deck to perform the "Acid Sterilization" phase. While it is possible to run this phase while the eDNA sampler is deployed, time constraints and difficulty maintaining station meant cleaning was done on deck during this deployment. Due to the abundance of material in the water column at 5 m, the sampler was deployed for the entire time the boat was on station (15-17 min). All samples from this deployment were stored inside the cassette overnight, after which they were removed and stored in a -20 $^{\circ}\text{C}$ freezer prior to DNA extraction. The 35 μm pre-filter was also preserved and extracted along with the sample membranes in order to ensure it was not affecting results.

During the transect, eDNA samples were also collected using a niskin bottle and peristaltic pump in order to compare results to the eDNA sampler. At each station, a 5 L niskin captured a water sample at 5 m, directly next to the eDNA sampler. Duplicates from a singular niskin cast were then filtered on deck using a peristaltic pump and boat battery. Between 660-1140 mL of water was filtered through 0.2 μm pore size, 47 mm diameter PC membranes (Millipore) for each duplicate and then immediately frozen in a cryoshipper primed with liquid nitrogen.

Table II outlines the DNA concentrations of the extracted samples (in 50 μL of AE buffer), the volume filtered across the membrane, and other metrics for all transect samples. Samples collected using the DOT eDNA sampler had consistently lower eluted DNA concentrations than those collected using the niskin and peristaltic pump, due to the different volumes

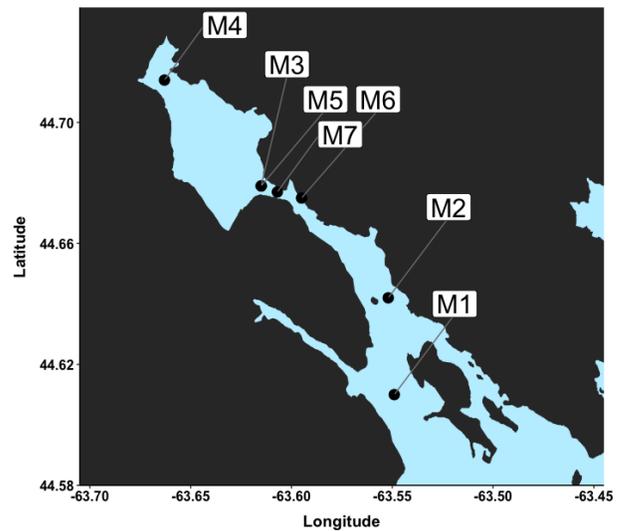


Fig. 5. Map showing the location of Halifax Harbour transect sampling locations. Samples are numbered in sequential order with M1 being first and M7 last. Samples M3 and M5 were taken at the same location approximately 2 hours apart.

of water filtered. The estimated DNA concentration of the original sample was determined from the mass collected on the membrane by using the volume filtered and the elution concentration. The original sample DNA concentration is comparable between the sampler and niskin samples as expected. As DNA extraction efficiencies are not 100% it is not expected that DNA concentrations would be identical.

We characterized the taxonomic composition of our samples as amplicon sequence variants (ASVs). Individual ASVs are clusters of highly related DNA sequences that are treated as a single homogeneous unit; each is assigned to a particular taxonomic group such as species, with multiple ASVs potentially mapping to the same group. A comparison of the top 10 most-abundant ASVs in each sample was performed via heatmaps for both bacteria (figure 6) and chloroplast (figure 7) ASVs. At all stations, the majority of top bacterial ASVs were the same between niskin and sampler samples (figure 6). Some bacterial ASVs occurred only in samples collected by the eDNA sampler and were therefore thought to be contaminants leftover in the lines. This includes *Ralstonia pickettii*, which was in the top 3 ASVs at station M1. However, when comparing all samples, the *Ralstonia pickettii* ASV is seen to decrease in relative abundance over time in sequential samples from the eDNA sampler (figure 6).

In the chloroplast 16S rRNA amplicon data, all samples were dominated by a bloom of Thalassiosirales that was picked up by both the niskin bottle captures and eDNA sampler (figure 7). Notably, the pre-filter sample does not share the same top bacterial ASVs with any sample from niskin or sampler (figure 6), and the one chloroplast ASV found in the pre-filter was not found in any other sample, niskin or sampler (figure 7). This indicates that the pre-filter did not filter out any significant organisms that were picked up in the niskin

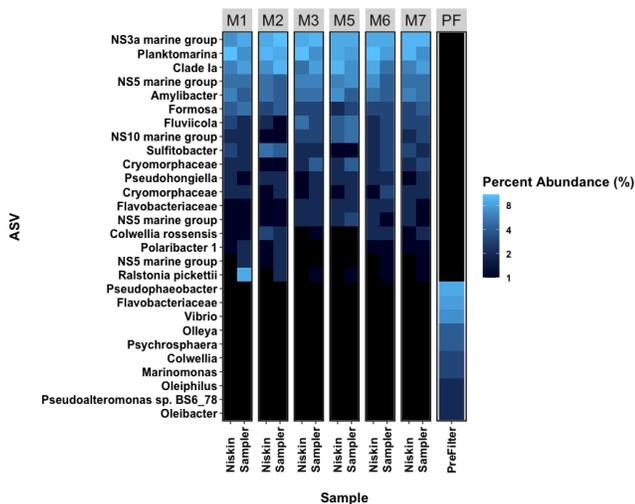


Fig. 6. Heatmap of the top 10 most-abundant bacterial ASVs in each sample and the pre-filter. Relative abundance is expressed as a percentage of the total bacterial ASVs from rarefied data. ASVs were classified down to the most-precise taxonomic rank possible.

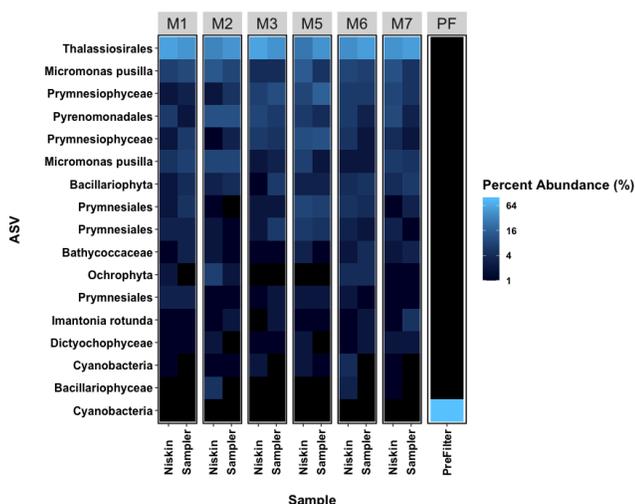


Fig. 7. Heatmap of the top 10 most-abundant chloroplast ASVs in each sample and the pre-filter. Relative abundance is expressed as a percentage of the total chloroplast ASVs from rarefied data. ASVs were classified down to the lowest taxonomic classification possible.

samples, and therefore did not affect results.

Looking further at the larger bacterial community in each sample, the relative abundance of the taxonomic classes is reasonably comparable between the eDNA sampler and niskin samples at each station (figure 8).

IV. DISCUSSION

When deployed in a real-world scenario off the side of a boat, the DOT eDNA sampler's performance was comparable to the traditional method of collecting water with a niskin and filtering the sample using a separate peristaltic pump aboard the vessel. Although the eDNA sampler's smaller filter and lower volume filtered meant that extracted samples had a lower DNA mass collected, this did not significantly impact the accuracy of the DNA sequencing results. Samples from both

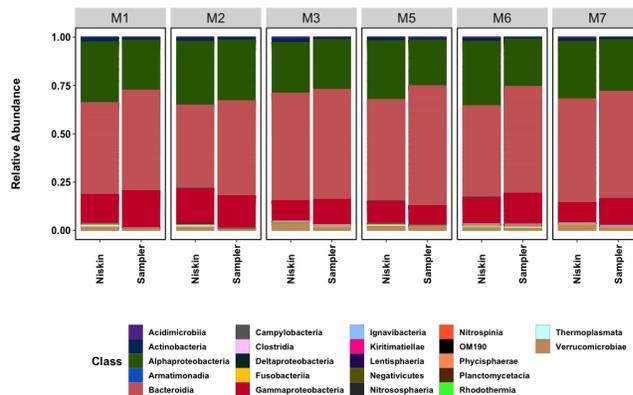


Fig. 8. Stacked bar plot showing the relative abundance of all bacterial classes at each sampling station for both niskin bottle captures and eDNA sampler captures. At all stations, the two methods show comparable relative abundance for the taxonomic classes.

the sampler and niskin had similar community compositions and relative abundances. This aligns with other studies which have found that sampling methods involving small sample volumes [47] as well as composite samples (samples taken over a period) did not differ significantly from larger volume or discrete samples (samples taken at a single point in time) [48].

Further evidence of this is seen in the heat map results which show that the majority of ASVs with high relative abundance were the same between the niskin and sampler samples at each station. As well, although there was some bacterial contamination in the sampler, these ASVs were, for the most part, not present at high relative abundance in the DNA samples. The exception was in the first sample collected at M1, where *Ralstonia pickettii* was present at a high relative abundance. *Ralstonia pickettii* is a species of bacteria known to form a biofilm on plastics and survive even in ultrapure water systems, therefore it is not surprising that it was found in the sampler's plastic lines [49]. The prevalence of *Ralstonia* in the M1 sample is likely because this was the first sample taken, and the sequential drop in contaminant reads in subsequent samples indicates that the current cleaning protocol was cleaning the lines over time. This suggests that once the cleaning protocol is optimized in future studies, bacterial contamination in the sampler will no longer be an issue.

One of the most noticeable differences between the sampler and the niskin methods is that the sampler is fitted with a 35 μm pre-filter on the inlet due to the particle limitations on the pump and valves, therefore the pre-filter was studied to ensure it was not retaining any important biological information. The pre-filter had the smallest DNA concentration of all the samples and the recovery of the DNA per volume filtered was 10-fold less than for the actual samples. DNA from the pre-filter had a different composition, and there were no ASVs present in the pre-filter that were recovered in the niskin samples, but not the eDNA sampler. Therefore, the pre-filter did not affect the sampler's results, and still allowed the sampler to pick up important aspects of the aquatic community,

TABLE II

DNA METRICS OF BOTH eDNA SAMPLER AND NISKIN SAMPLES AT EACH STATION. DNA IN THE ORIGINAL SAMPLE WAS BACK CALCULATED FROM THE CONCENTRATION OF ELUTED DNA USING THE ELUTION VOLUME (50 µL FOR ALL SAMPLES).

Station	Method	Sample Volume (mL)	Eluted DNA (ng/µL)	DNA in Sample (ng/mL)	260/280	Number of Raw Reads
M1	Sampler	125	8.3	3.3	2.23	37081
	Niskin	760	97.3	6.4	1.91	5511
M2	Sampler	125	4.9	2.0	2.05	46863
	Niskin	740	57.8	3.9	1.9	5404
M3	Sampler	125	10.6	4.2	2.15	30270
	Niskin	770	93.8	6.1	1.86	11839
M5	Sampler	125	16.4	6.6	2.05	20218
	Niskin	660	55.1	4.2	1.89	6901
M6	Sampler	125	4.7	1.9	2.27	10675
	Niskin	880	35.5	2.0	1.92	8972
M7	Sampler	125	9.4	3.8	2.15	12667
	Niskin	1140	68.5	3.0	1.92	14000
Pre-filter	Sampler	875	3.1	0.2	2.48	25100

as shown by the dominance of the blooming *Thalassiosirales* at the time of sampling.

Overall, these results demonstrate a successful first in-field deployment of a newly developed automated eDNA sampler. The sampler performed in a comparable manner to the commonly used niskin collection and separate filtration approach and, with future development will be a convenient alternative to traditional sampling methods, making eDNA sampling more accessible to the broad community employing eDNA in diverse applications.

V. CONCLUSION

This paper demonstrates a novel eDNA sampler that integrates cleaning, preservation, and a field-swappable cartridge. The filter cartridge can be changed in 5 minutes for quickly reloading filters during deployment; useful for AUV missions or for traditional rosette casting operations when collecting eDNA samples. The sampler was deployed along the Bedford Basin and a direct comparison to traditional niskin sampling was performed to ascertain the eDNA sampler's performance. At all 6 stations sampled, the two methods showed comparable taxonomic compositions, demonstrating the viability of the sampler. Ongoing work will quantitatively characterize cross-contamination and expand the field deployment scenarios for increasing the readiness of the instrument. We believe DOT's eDNA sampler is an enabling technology for the collection of samples, particularly for use in remote locations and for extended durations, such as biodiversity monitoring programs of Marine Protected Areas.

CONFLICT OF INTEREST

Arnold Furlong, Julie LaRoche, Robert Beiko, and Vincent Sieben declare share holdings in DOT Inc.

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