APPLICATION

eDNA Sampler: A fully integrated environmental DNA sampling system

Austen C. Thomas¹ | Jesse Howard¹ | Phong L. Nguyen¹ | Tracie A. Seimon² | Caren S. Goldberg³

¹Smith-Root, Vancouver, WA, USA  
²Wildlife Conservation Society, Zoological Health Program, Bronx, NY, USA  
³School of the Environment, Washington State University, Pullman, WA, USA

Correspondence  
Austen C. Thomas  
Email: athomas@smith-root.com

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Abstract

1. Species monitoring using environmental DNA (eDNA) is a powerful new technique for natural resource scientists and the number of research groups employing eDNA detection is growing rapidly. However, current eDNA sampling technologies consist mainly of do-it-yourself solutions, and the lack of purpose-built sampling equipment is limiting the efficiency and standardization of eDNA studies.

2. Here, we describe the first fully integrated sampling system (Smith-Root eDNA Sampler) designed by a team of molecular ecologists and engineers for high-throughput eDNA sample collection. It consists of a backpack portable pump that integrates sensor feedback, a pole extension with remote pump controller, custom-made filter housings in single-use packets for each sampling site and onboard sample storage. The system is optimized for sampling speed and replicability, while minimizing risk of contamination.

3. We present an example pilot study designed to identify optimal eDNA Sampler system parameter values (i.e. pump pressure, flow rate, filter pore size, sample volume) in a new sampling environment. We identified a peak in filtration efficiency at a flow rate threshold of 1.0 L/m, and found that 5 μm filters captured significantly more target eDNA than 1 μm filters. Results also suggest that high filtration pressures may reduce eDNA retention, which implies that pressure should be standardized to avoid biasing detection data.

4. Similar to the technological evolution of backpack electrofishers, eDNA sampling technology is in the process of transitioning from a nascent phase to professionally engineered research tools. Such innovations will be essential as eDNA monitoring becomes one of the industry standard methods used for species detection and management.

KEYWORDS  
community ecology, disease ecology, invasive species, monitoring, population ecology, sampling

[Correction added on 29 June 2020, after first online publication: the article title has been corrected and all instances of ANDe have been changed to eDNA Sampler throughout the article and Supporting Information files.]
1 | INTRODUCTION

Surveys of aquatic species are necessary for effective resource management, often contributing to the early detection of invasive organisms, stock assessments and the detection of rare or endangered species (Ricciardi et al., 2017). However, management actions stemming from the information gathered in aquatic species surveys are only as good as the data on which they are based (Guisan et al., 2013). It is thus incumbent upon the scientific community to continuously refine existing survey techniques and/or develop new methods to ensure that our resource managers have the best possible information on which to base management decisions.

A new transformational survey technology is now available to aquatic researchers in the form of environmental DNA (eDNA) monitoring (Rees, Maddison, Middleditch, Patmore, & Gough, 2014). Relatively recently, it was discovered that eDNA in aquatic environments can be used to detect vertebrate species without necessitating invasive physical contact with target organisms (Ficetola, Miaud, Pompanon, & Taberlet, 2008). Since then the field has grown rapidly as ecologists and managers have applied these methods to address long-standing challenges in invasive and rare species detection (Rees et al., 2014). The range of eDNA applications now extends well beyond targeted detection of aquatic species, and includes terrestrial animal detection from water samples (Williams, Huyvaert, Vercauteren, Davis, & Piaggio, 2018), in addition to biodiversity assessment via eDNA metabarcoding methods (Valentini et al., 2016).

eDNA sampling methods, however, are still under development (Goldberg et al., 2016). Some protocols require samplers to transport volumes of water back to the laboratory, which limits sample size and introduces the possibility of DNA degradation before filtration or preservation can be performed (Goldberg et al., 2016). In addition, field-filtration methods that circumvent the problems of water transport often rely on tools designed for other processes, making sampling efficiency and cross-contamination considerations ongoing challenges. It is an opportune time for eDNA sampling equipment to make the transition from do-it-yourself solutions to fully engineered, purpose-built, ecological research tools.

Here, we describe the development of a novel backpack eDNA filtration system designed to improve the efficiency, sterility and replicability of aquatic eDNA sampling for field users (the Smith-Root eDNA Sampler system). We elaborate upon our system design considerations and how this new technology can be used to improve the outcomes of eDNA detection studies. A video tutorial demonstrating the eDNA Sampler system workflow is available online (Video S1, www.smith-root.com/support/tutorials).

2 | SYSTEM DESIGN AND RATIONALE

The first challenge was to design a system that minimizes the risk of sample contamination in the field, wherein the user is not required to directly enter the water (unless conducting transect/continuous sampling) and the only components of the system that contact the water are sterile (i.e. devoid of contaminating target DNA). Our solution was to create a negative-pressure inline filtration system that places a single-use, eDNA concentrating filter housing on the upstream end of tubing, making the components downstream of the filter reusable between samples. The filter is mounted at the end of a pole extension, keeping the user away from the location being sampled (Figure 1). This solution minimizes the amount of single-use plastic waste per sample or sterilization effort, while also minimizing the risk of contaminating DNA contacting the filter.

A wide range of filter pore sizes and membrane materials are currently used to achieve optimal eDNA capture (Rees et al., 2014). Therefore, the next challenge was to create an inline filter housing that can be used with many different types of filters and can be opened in the field to remove and preserve the filter. We engineered a custom, low-cost eDNA filter housing that is compatible with any 47 mm filter membrane and is openable via a pull-tab. The filter housings were engineered for flow characteristics that minimize large particulate accumulation and maximize eDNA filtration rate (Figure 2). This is achieved using a combination of large inlet aperture, inverted filter housing orientation and reduced flow velocity at

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**FIGURE 1** Illustrating two types of environmental DNA sampling using the eDNA Sampler system: (a) point sampling, using the long pole extension and support bipod to minimize risk of contamination to sampled water and (b) continuous sampling, whereby the user walks along a transect with the pump set to a constant flow rate to standardize sampling over distance.
the filter interface which allows heavier particles to fall out while the finer particles are retained. These filter housings can be preloaded by the manufacturer with alternative membrane materials and combined with other single-use consumables (e.g. forceps, tubing extension) in a sealed packet for each sample.

Our next objective was to create an intelligent pump system for the eDNA sampler that can regulate filtration based on information gathered by external sensors, each monitoring a factor that influences eDNA capture (e.g. flow rate and filtration pressure). The goal was to give users control over these influential factors by integrating user-input thresholds and/or set-points for the specific variables. This is critical for optimization of eDNA filtration and standardization of sample collection.

Currently, little is known about the material properties of eDNA or the effects of alternative sampling strategies on eDNA capture; although, size fractionation experiments indicate that eDNA is most likely bound in free-floating cells or cell clumps, as opposed to “unprotected” exogenous DNA in the water column (Turner et al., 2014). We thus hypothesized that extreme filtration pressures likely impact the integrity of cells or cell clumps containing eDNA (possibly

**FIGURE 2** A typical eDNA Sampler system workflow for environmental DNA (eDNA) sampling, including: (1) powering on the system inside the waterproof compartment, (2) programming the user-input parameters (sample volume, pressure and flow limits), (3) removing a sample packet containing pre-loaded filter housing, forceps and tubing extension, (4) mounting the filter housing to the pole extension clamp, (5) activating the pump using the remote control on the pole extension, (6) water filtration while monitoring the volume filtered display on remote control, (7) opening of the filter housing once filtration is completed, (8) preservation of the eDNA filter membrane in ethanol or alternative preservative and (9) storage of collected samples inside the waterproof compartment.
by causing cell rupture), and by extension could affect retention of eDNA on filter membranes. We therefore chose to include a pressure sensor in the system, allowing the user to input a pressure threshold that the system is designed to not exceed during the filtration process.

A flow sensor and user-input flow threshold were also integrated into the system for two reasons: (a) to facilitate transect/continuous sampling whereby the user can sample over distance at a constant flow rate while moving through the sampled environment (Figure 1) and (b) to optimize flow dynamics for maximal water particle concentration on a single filter. The concept of continuous sampling for eDNA (as opposed to point sampling) has not yet been broadly applied in research studies, mainly due to logistical constraints of existing pump systems. The eDNA Sampler system is built to overcome these constraints by improving pump portability and allowing the user to precisely control flow and pressure.

Lastly, the eDNA sampling system needed to be compact and capable of containing all the equipment and supplies required to collect many samples in remote locations and in inclement weather. Building on the geometry of the LR-20B backpack electrofisher, the diaphragm pump, circuit boards, sensors and sample storage area are contained inside a water-tight fibreglass box. External to this is an interchangeable 12 V lithium-ion battery, a pair of zippered dry bags for sample packet storage and a carbon fibre collapsible pole extension. The pole extends up to 3.6 m and is supported by a telescoping bipod, helping the user control the end of a relatively long sampling extremity. Mounted to the pole is a waterproof remote controller for the pump system with wireless communication that allows the user to activate or deactivate the pump while managing the pole extension and monitoring the amount of water filtered.

System cost information can be found online (www.smith-root.com).

3 | IMPLEMENTATION

The eDNA Sampler system setup and the typical sampling workflow is depicted in Figure 2. A Quick Start Manual can also be found in Supporting Information (Manual S2).

When beginning a study, the user should evaluate settings of the three user-input parameters (1—sample volume, 2—pressure threshold and 3—flow threshold) and filter pore size. We recommend starting with an in-situ experiment with a known presence of the target organism in water that is representative of the sampling environments. Sample volume, pressure threshold, flow threshold and filter pore size can then be manipulated to identify optimal values for eDNA capture (using target DNA copy number as the response variable). Optimal values are likely to vary depending on environmental conditions and the target species taxon.

The user can select from either auto-mode or manual-mode using the remote controller. If set to auto-mode, the pump system will alert the user when the desired sample volume has been reached (at which point the filter should be inverted), and pumping should continue until the system registers a low-flow alarm (indicating that all filtered water has been totalled). In manual-mode, the user can start and stop the pump at will, and the system will tally the total volume filtered while maintaining pressure and flow within parameter specifications. The low-flow alarm is also useful as an indication of filter clogging and can be used to determine a realistic sample volume.

Once filtration is complete, the pole is retracted and stabilized for filter membrane removal. A preloaded vial with 95% ethanol (or alternative preservative) should be prepared and readily available. Next, the seal of the filter housing is broken by the user pulling the thumb tab, separating the two halves and exposing the filter. Working with the single-use forceps provided in the sample pack, the user then carefully folds the filter membrane and inserts it into the ethanol-filled vial for preservation. Other methods of preservation (e.g. filter envelope and desiccant storage) or immediate on-site DNA extraction are also possible at this stage. Finally, the sample ID and all necessary metadata (e.g. environmental variables) are recorded for each sample using a tablet computer or notebook. The user then proceeds to the next sampling location.

4 | PILOT STUDY EXPERIMENTS

To demonstrate the advantages of working with the eDNA Sampler system and to illustrate the optimization process for a new sampling environment, we conducted two small experiments.

4.1 | Flow experiment

A common objective in eDNA studies is to maximize the amount of water that can be filtered as a means of increasing the likelihood of species detection. Working in a natural river environment (Salmon Creek, Washington, USA), we tested the effects of user-input flow rate on the total filterable volume, by varying the threshold value and measuring the volume of water filtered prior to the system registering a “low-flow” state (flow ≤ 0.1 L/min). Three replicate filter samples were taken for each of five flow rate thresholds (0.4, 0.6, 0.8, 1.0 and 1.2 L/min), and two mixed cellulose ester filter pore sizes (1 and 5 μm). The experiment was conducted on a day with low suspended particulate in the water, and then repeated after a rain event which created elevated levels of suspended particulate in the river.

4.2 | Pressure experiment

To our knowledge, the impact of filtration pressure on eDNA retention and detection is not currently known; however, we hypothesized that pressure is an influential factor based on limited published data regarding eDNA physical properties. We tested this hypothesis by quantifying eDNA collected on filters subjected to different filtration pressures. Water (130 L) was collected from the aforementioned river and placed in a circulated tank designed to maintain suspended particles. To this tank, we added an additional 4.0 L of water obtained from a 38 L rearing tank containing a population
Three replicate filter samples were taken for each of five pressure thresholds (6.0, 8.0, 10.0, 12.0 and no limit -psi), and two filter pore sizes (1 and 5 μm) with flow rate standardized for all samples (1.0 L/min). For reference, 0.0 -psi = 1 atm. As with the flow experiment, filtration was stopped when the filter became clogged and the system registered a "low-flow" status (flow ≤ 0.1 L/min).

Filter samples were preserved in ethanol, and mudsnail eDNA was detected using a standard qPCR protocol (Goldberg, Sepulveda, Ray, Baumgardt, & Waits, 2013) and quantified via Cq comparison to a gBlock® standard curve. Further MIQE parameters can be found in Document S3. We used these data to calculate total mudsnail eDNA per filter sample (copies per filter) following the equation:

\[
eDNA_{\text{Total}} = \left( \frac{SQ}{T_{\text{vol}}} \right) \cdot E_{\text{vol}} \cdot F_p^{-1},
\]

where SQ is the calculated starting quantity (DNA copies per reaction) based on the standard curve, \(T_{\text{vol}}\) is the volume of template DNA (μl) included in the qPCR reaction, \(E_{\text{vol}}\) is the elution volume of the filter DNA extraction process (μl) and \(F_p\) is the proportion of the 47 mm filter that was used for the extraction (in this case 0.5). eDNA captured per litre of water filtered (copies per L) was then simply calculated as:

\[
eDNA/L = \frac{eDNA_{\text{Total}}}{F_{\text{vol}}},
\]

where \(F_{\text{vol}}\) is the volume (L) of environmental water filtered for the particular sample. To each of these response variables, we applied a two-way ANOVA test, using filter pore size and pressure threshold value as predictor variables. Figure error bars indicate the standard error of three replicate filter samples.

\[5 \, \text{RESULTS/DISCUSSION OF PILOT STUDY}\]

A representative filtration profile displaying both pressure and flow data during the sample filtration process is depicted in Figure S4, and consists of four distinct phases (see figure caption for details).

The flow experiment yielded several results that may provide a useful starting point for eDNA Sampler system users. Manipulation of the flow rate threshold value had a substantial impact on the volume of water that can be filtered prior to clogging (Figure 3). The average volume filtered by 1 μm filters at a flow rate threshold of 1.0 L/min (3.0 L) was over twice the volume filtered at a flow rate threshold of 0.4 L/min (1.4 L). Furthermore, the average volume decreased when the flow rate threshold reached 1.2 L/min, indicating a clear peak in filtration efficiency at 1.0 L/min. Also interesting was the observation that 5 μm filters in high particulate water responded similarly to flow threshold manipulation compared to 1 μm filters in low particulate water.

Our pressure experiment results indicate a competing relationship between the amount of water that can be filtered at a given pressure and eDNA retention on the filter surface (Figure 4). For example, mudsnail eDNA per litre of water filtered decreased by 39% when the filtration pressure increased (max pressure). There was weak evidence that pressure as a factor was influential with this small sample \((F_{6,24} = 2.291, p = .089)\), suggesting that for every unit of water filtered, less eDNA is retained on the filter at high pressures than it is at lower pressures (potentially due to lysis of cells at high negative pressure). However, the reduction in eDNA retention is apparently offset by increased filterable water volume at high pressures (e.g. moving from 6.0 to 12.9 –psi increased the water...
volume filtered from 0.79 to 1.4 L per filter, an 81% increase). This explains the lack of a relationship between filtration pressure and total mudsnail eDNA per filter \( F_{(4,24)} = 0.531, p = .714 \), because total eDNA is a function of both eDNA retention and total volume.

Lastly, counter to the large number of eDNA studies employing small pore sized filters (0.45–1.0 μm) (Rees et al., 2014), we found that 5 μm filters yielded significantly more total mudsnail eDNA than 1 μm filters \( F_{(1,24)} = 4.359, p = .048 \), despite our small sample size (Figure 4). The combination of larger filterable water volume prior to clogging and comparable eDNA retention in this experiment made 5 μm filters superior to 1 μm for total eDNA capture.

Based on these results, we recommend using a flow threshold value of 1.0 L/min when filtering water volumes <3.0 L, and a flow threshold of 0.8 L/min for larger volumes. If the eDNA Sampler system is unable to prime (water does not reach the pump due to high particulate load) with 1 μm filters, we recommend increasing the pore size to 5 μm and concentrating a larger water sample. Our data suggest that 5 μm filters may be overall more efficient for eDNA capture, regardless of particulate load, if volume filtered is maximized. In studies that target a specific sample volume for standardization purposes (e.g. 1.0 L), we suggest using the minimum filtration pressure required to achieve that sample volume, which should maximize eDNA retention. Most importantly, the filtration pressure should be standardized when sampling fixed volumes to avoid variability in eDNA retention that could bias species detection results.

6 | CONCLUSION

Given the rapid expansion of eDNA as a species monitoring tool, it is likely that eDNA sampling will soon become one of the fundamental
techniques in the ecologist’s toolbox. The eDNA Sampler system is the first of potentially many future instruments specifically designed to increase the efficiency and replicability of eDNA sample collection. This is the beginning of an exciting era of new molecular technologies that will ultimately be refined and further developed by the eDNA user community.

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AUTHORS’ CONTRIBUTIONS
A.T. conceived the idea for the eDNA sampling system; A.T., J.H. and P.L.N. were principally involved in the design process; A.T., P.L.N., T.S. and C.G. conceived of and produced the experimental data; A.T. wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

DATA ACCESSIBILITY
The following data files have been archived in Dryad Digital Repository. Pressure_Data.csv—Data produced from the flow experiment. qPCR_Data.csv—qPCR results from the pressure experiment for each individual qPCR replicate (Thomas, Howard, Nguyen, Seimon, & Goldberg, 2018).

ORCID
Austen C. Thomas http://orcid.org/0000-0002-6182-9440
Tracie A. Seimon http://orcid.org/0000-0003-3658-1364
Caren S. Goldberg http://orcid.org/0000-0002-0863-9939

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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