Title: Innovations in eDNA Sampling Technology: comparing manual and automated techniques

Authors: Connelly F[1*], Weeks AR[1,2], Tingley R[3], and Berran M[3]

[1] EnviroDNA, 293 Royal Pde, Parkville VIC, 3052. Email: fconnelly@envirodna.com
[2] School of Biosciences, University of Melbourne, Melbourne, VIC
[3] School of Biological Sciences, Monash University, Clayton, VIC

Key Points

- Automated Smith-Root samplers were able to process larger volumes of water
- Automated Smith-Root samplers were more effective at single species detection
- Automated Smith-Root samplers and the manual technique performed similarly at multispecies detection

Abstract

Environmental DNA (eDNA) is a rapidly growing technique used to detect individual or groups of species using DNA fragments in environmental samples, primarily water. Early applications of eDNA typically involved filtering water samples in a laboratory or manually forcing water through a filter in the field. However, new innovative technologies have automated this process. Smith-Root Inc. has created automated sampling systems that allow the filtering of large volumes of water with speed and efficiency, including larger water bodies such as lakes, reservoirs and marine systems. These systems have the potential to optimise eDNA sampling; however, the efficacy of these new pumps compared to manual filtering has yet to be investigated. In this paper, we detail the results of two experiments that compare the effectiveness of both the automated and manual techniques at detecting species through eDNA. Each experiment utilises a different eDNA analysis, the first metabarcoding or multispecies analysis, and the second target or single species analysis. Our results indicated that the automated sampling system was able to filter a larger volume of water and was better at target species detection as compared to the manual method; however, both methods were equally effective at multispecies detection.

Keywords
Environmental DNA, Smith-Root, Sterivex, metabarcoding, qPCR, filtration, biodiversity

Introduction

Environmental DNA (eDNA) refers to DNA shed by an organism into the environment. eDNA can be detected from a sample (i.e., water, soil, and air) without the organism of interest being present at the exact moment of collection (Goldberg et al. 2016). The use of eDNA sampling is a rapidly evolving and immerging as an efficient, non-invasive method for detecting organisms (Muha et al. 2019). It is especially useful when surveying for elusive or rare species that occur in low abundance or species often difficult to observe using traditional methods (i.e., point counts or live trapping; Zhang et al. 2020). Currently eDNA can be conducted in two different ways: 1. Target or single species assays; 2. Metabarcoding or multispecies assays (within the same taxa, i.e., vertebrates or fishes; Harper et al. 2018). While the scope of target species analysis is limited to a single species, metabarcoding offers a cost-effective approach for conducting large scale, community-wide, biodiversity studies, that have been shown to be more effective than previous biodiversity assessment methods (Smart et al. 2015; Hänfling et al. 2016; Bálint et al. 2018; Eiler et al. 2018; Lugg et al. 2018; Takahara et al. 2020).

The most common technique used for eDNA collection is via the passing of water through a filter (Spens et al. 2017). The effectiveness of this method is well documented and allows for the processing of large volumes of water and therefore large quantities of eDNA (Goldberg et al. 2016, 2018; Muha et al. 2019). Variation between methods is seen throughout the literature, indicating there are differences in detectability of eDNA depending on filter pore sizes and the volume of water that can be processed (Turner et al. 2014; Schabacker et al. 2020). Smith-Root Inc. created an automated eDNA sampling system that has the potential to challenge the more commonly used manual sampling technique. There is, however, no current literature comparing automated and manual techniques. In this study, we investigate how two different eDNA sampling techniques (manual or "Sterivex" and automated or "Smith-Root") compare. Using data from two different experiments, we explore how these two techniques compare by looking at the total volume of water they can process, their ability to filter in varying levels of turbidity, and their effectiveness at detecting eDNA using both target species (an amphibian species) and multispecies assays.

Methods

Study area and site descriptions

The data for this paper was compiled from two different projects conducted by EnviroDNA between 2020 – 2021. Water samples were collected from various waterways across Australia. Waterbodies included flowing water (creeks, and rivers), wetlands, and larger water bodies including lakes and reservoirs. Specific location details were not included as per our non-disclosure agreements with our clients.

Water Sampling Techniques

Sterivex: water samples were collected and filtered *in situ* by passing water through a 0.22 μm filter (Sterivex) using a sterile 60 ml syringe (Figure 1a).

Smith-Root: water samples were collected using an ANDe™ eDNA Sampler Backpack (Smith-Root Inc, Vancouver WA, US; Figure 1b). Smith-Root samplers are fully integrated sampling systems consisting of a remote controlled portable pump with sensor feedback (pump pressure, flow rate, and sample volume; Thomas et al 2018). The pumps utilise Smith-Root self-preserving filters that automatically preserve the samples via desiccation (Thomas et al 2019; Figure 1c). Two filter pore sizes were used in the experiments – 1.2 μm and 5.0 μm.

The volume of water filtered with both the Sterivex and Smith-Root filters varied between the two experiments and between sites (Sterivex: 15 - 400mL; Smith-Root 1.2 μm and 5.0 μm: 80 - 1700mL &

44 - 5400mL respectively); volumes were dependent on water conditions including turbidity. Care was taken to minimise contamination between sites through the use of clean equipment and gloves at each site and by avoiding the transfer of water, soil, or organic matter between sites.

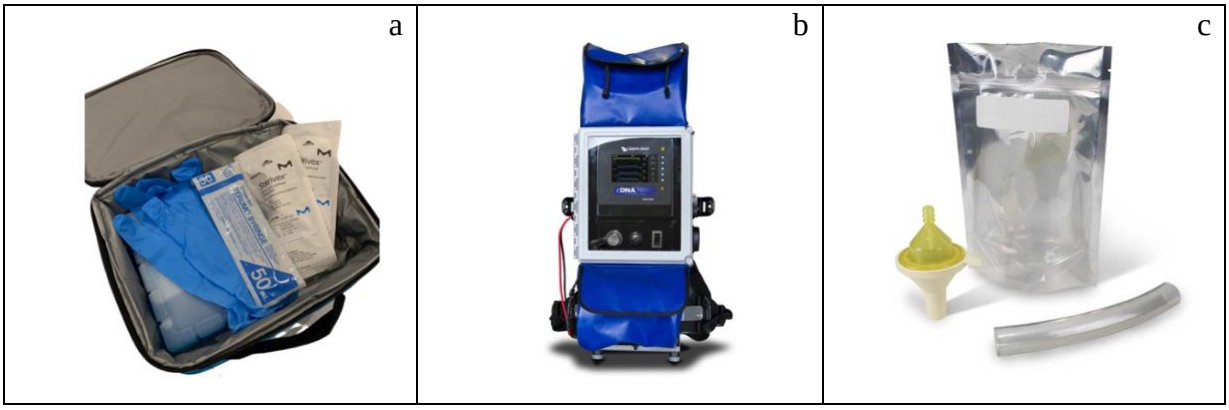

Figure 1. eDNA filtering methods used: a) EnviroDNA eDNA kit containing Sterivex filters, 60mL syringe, and gloves; b) Smith-Root ANDe™ eDNA Sampler Backpack; c) Smith-Root self-preserving filters.

Experiment A: *Comparing Sterivex and Smith-Root - volume, turbidity and metabarcoding*

Sterivex: At each site (n = 25), five Sterivex samples were collected at different sampling locations around the waterbody resulting in five independent Sterivex filter samples per site. All Sterivex filters were kept cool and out of direct sunlight prior to freezing at the end of each sampling day.

Smith-Root: 1.2L of water was collected from the same five sampling locations where Sterivex filters were collected. The five samples were consecutively pooled into a DNA sterile bucket to form one 6L pooled water sample. The Smith-Root sampler was then used to pump up to 6L of water through a single 5.0 µm self-preserving filter from each bucket, resulting in one Smith-Root filter sample per site. Filters were kept cool and protected from direct sunlight prior to DNA extraction.

Three turbidity readings were measured at each sampling location and from each bucket of pooled water using a portable digital turbidity meter (0 - 1000 TNU); the average was calculated for each location/bucket. We measured turbidity at each site/bucket as high turbidity may limit sample volumes and possibly affect eDNA yield (Bedwell and Goldberg 2020).

Experiment B: *Comparing Sterivex and Smith-Root (2 pore sizes) – volume and target species (Amphibian sp.)*

At each site (n =10), four water samples were processed through Sterivex filters, two through the 1.2 µm Smith-Root filter and one through the 5.0 µm Smith-Root filter. Filters were kept cool and protected from direct sunlight prior to DNA extraction.

DNA processing and analysis

Extraction: DNA was extracted from both filter types (Sterivex and Smith-Root) using a commercially available DNA extraction kit (Qiagen DNeasy Blood and Tissue Kit) and samples were analysed using either a real-time quantitative polymerase chain reaction (qPCR) approach (amphibian sp.) or a metabarcoding approach (vertebrate biodiversity).

Target species assay: A species-specific marker spanning part of the mitochondrial tRNA-Gly and NADH dehydrogenase subunit 3 (ND3) genes of the amphibian sp. mitochondrial genome (Tingley et al 2019) was used to detect the amphibian sp. eDNA. Real-time qPCR was carried out on samples to amplify the target DNA using a TaqMan Gene Expression Assay (Thermo Fisher Scientific). All samples were screened in triplicate along with a cane toad genomic DNA control. Negative controls were included at both the sampling, extraction and qPCR stages so that field and laboratory contamination could be identified if present.

Metabarcoding: Multispecies assessments were performed with a vertebrate assay targeting a small region of the mitochondrial DNA. Library construction involved two rounds of PCR whereby the first round employed gene-specific primers to amplify the target region and the second round incorporated sequencing adapters and unique barcodes for each sample-amplicon combination included in the library. Negative control samples were also included during library construction.

Statistical Analysis

Linear regressions were used to explore relationships between each continuous response variable and one or more predictor variables. All statistical tests used $\alpha = 0.05$ and all analysis was conducted using R Studio (version R-3.6.1; R Core Team 2019).

Results

Experiment A: *Comparing Sterivex and Smith-Root - volume, turbidity and metabarcoding*

The volume of water processed by each filter differed significantly between filter types (Figure 2). A single Smith-Root 5.0 µm filter processing over four times more water on average (mean = 763.13 mL ± 641.83 SD) than a single Sterivex 0.22 µm filter (mean = 177.45 mL ± 125.78 SD). The turbidity (logged) of the sampled water ranged from -0.42 to 5.61 TNU across all sampling locations and pooled samples. A significant negative correlation was observed between log turbidity and sample volume per filter for both filter types (Figure 2). Smith-Root filters were able to filter larger volumes of water as compared to Sterivex in similarly turbid water up until the most turbid samples (~4 TNU). The Smith-Root method (single filter from pooled water) performed similarly to the Sterivex method (5 filters from 5 locations) at multispecies detection (Figure 3), both techniques detecting a similar number of species.

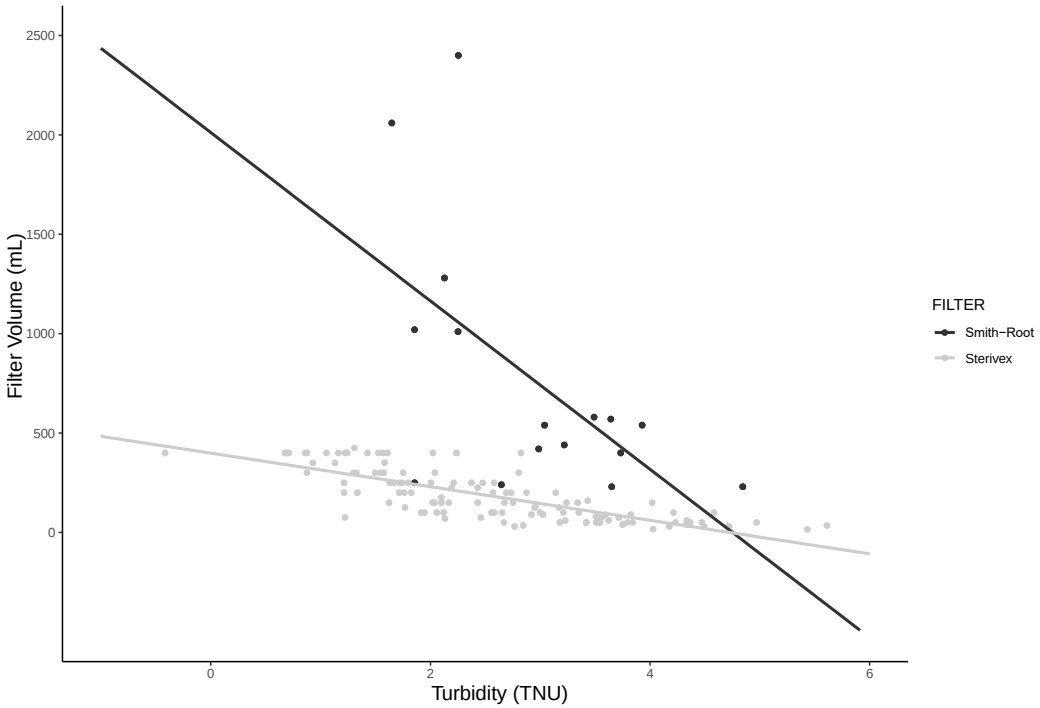

Figure 2. The relationship between log turbidity (TNU) and the volume of water processed per individual filter (mL) for each filter type: Smith-Root 5.0 µm and Sterivex 0.22 µm. Lines of best fit are shown.

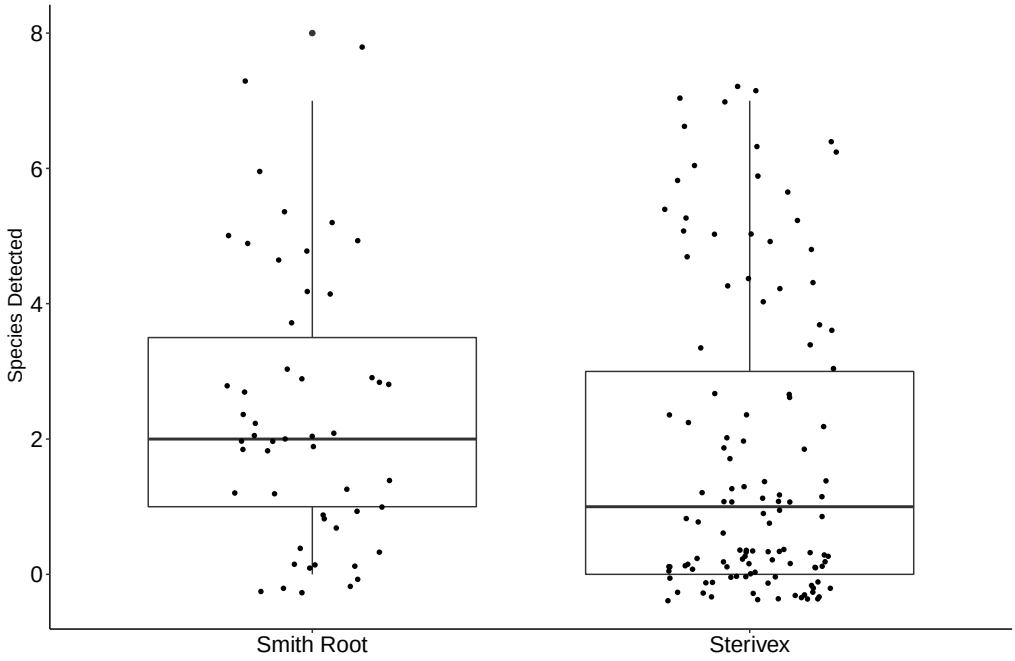

Figure 3. The number of species detected (species richness) for each filtration approach: Smith-Root (species richness of one pooled 5.0 µm filter sample per site), and Sterivex (species richness of five 0.22 µm filters per site).

Experiment B: *Comparing Sterivex and Smith-Root (2 pore sizes) – volume and target species*

Smith-Root performed better at single species detection compared to Sterivex. This was true for both the 1.2 µm and 5.0 µm filters (Figure 4). In addition, there were no differences in detectability between the 1.2 µm and 5.0 µm filters despite using two 1.2 µm filters and only a single 5.0 µm filters per site (Figure 5). Overall, Smith-Root filters (1.2 µm and 5.0 µm) were able to filter over 5L of water compared to the just 500mL using Sterivex.

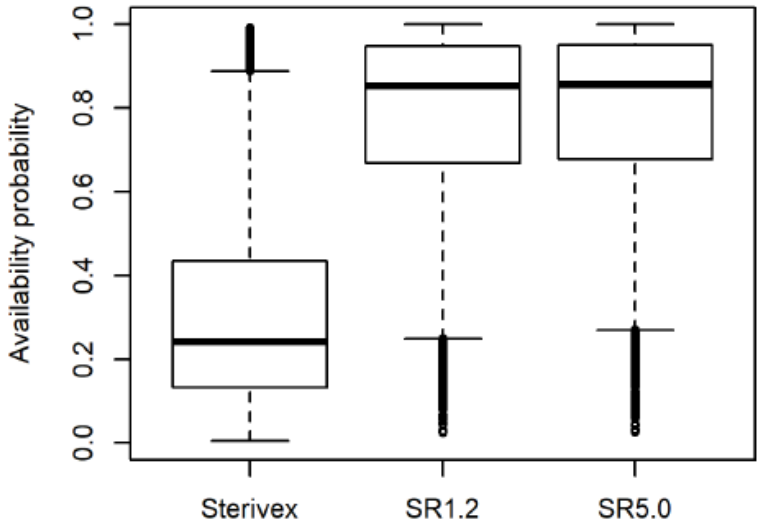

Figure 4. Predicted probabilities of detecting amphibian sp. eDNA in a water sample as a function of filter type at a single site. Sterivex (n=4), Smith-Root 1.2 µm (n=2) and Smith-Root 5 µm (n=1).

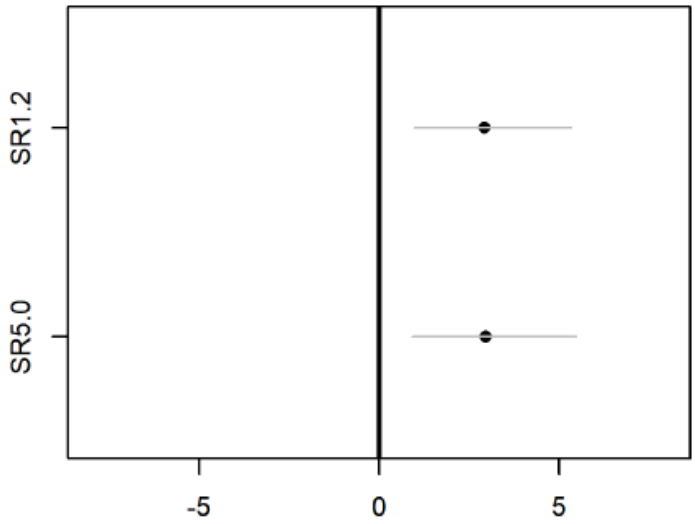

Figure 5. The mean and 95% credible intervals for the two filter type coefficients.

Conclusions

In this paper, we provide the results of two experiments that compare the effectiveness of two eDNA sampling techniques. We found that differences do exist between the two sampling techniques. The automated Smith-Root sampler was able to process larger volumes of water regardless of pore size (1.2 μm and 5.0 μm) compared to a single, manually processed Sterivex filter; in addition, Smith-Root performed better in turbid water. Smith-Root also performed better than Sterivex in target species detection; a single 5.0 μm and two 1.2 μm Smith-Root filters outperformed four Sterivex filters in detecting an amphibian sp. However, in multispecies detection, a single Smith-Root filter performed similarly to five Sterivex filters. This potentially indicates that while total water volume may be better for detecting a single species, it may not be as important when looking at multiple species. Some literature suggests that smaller filter pore sizes, such as the Sterivex 0.22 μm filter, are better at capturing eDNA (Turner *et al.* 2014; Eichmiller *et al.* 2016; Majaneva *et al.* 2018); however, our study shows this is not always true. The larger pore sizes found in the Smith-Root filters performed similarly to smaller pored Sterivex filters in multispecies assays and performed better at target species detection. Overall, while it did not outperform Sterivex in every comparison, we provide evidence that the automated Smith-Root sampler is a more efficient sampling method compared to the manual Sterivex technique.

It is important to note that only two sampling methodologies and two analyses (a single target species and a single species group) were used in this study; therefore, different strategies could yield alternative results. Future studies should focus on trialling different sampling methodologies using filters of different pore sizes, paired with analyses that investigate a variety of different target species and species groups. There is no 'one size fits all' approach to eDNA sampling in water and you must choose a sampling method based on what you are looking to detect (i.e., target species or multispecies) and the conditions of the water you are sampling (i.e., flowing water, large bodies of water, or turbid waterways). The use of eDNA to detect species presence and community biodiversity is constantly evolving, with new methods being developed each year. It is critical that the effectiveness of these new techniques are analysed and compared with previously proven methods to establish best practices. The two techniques compared in this study, Sterivex and Smith-Root, are both viable tools for eDNA collection and should continue to be used.

Acknowledgments
This research was funded in part by Melbourne Water.

References

Bálint M, Nowak C, Márton O, Pauls SU, Wittwer C, Aramayo JL, Schulze A, Chambert T, Cocchiararo B, Jansen M (2018) Accuracy, limitations and cost efficiency of eDNA-based community survey in tropical frogs. *Molecular Ecology Resources* 18, 1415-1426.

Bedwell ME, Goldberg CS (2020) Spatial and temporal patterns of environmental DNA detection to inform sampling protocols in lentic and lotic systems. *Ecology and Evolution* **10**, 1602-1612.

Eichmiller JJ, Miller LM, Sorensen PW (2016) Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish. *Molecular*

Eiler A, Löfgren A, Hjerne O, Nordén S, Saetre P (2018) Environmental DNA (eDNA) detects the pool frog (*pelophylax lessonae*) at times when traditional monitoring methods are insensitive. *Scientific Reports* 8, 5452-5459.

Goldberg CS, Strickler KM, Fremier AK (2018) Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of sampling designs. *Science of the Total Environment* 633, 695-703.

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-Mccance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder R (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods in Ecology and Evolution* 7, 1299-1307.

Hänfling B, Lawson Handley L, Read DS, Hahn C, Li J, Nichols P, Blackman RC, Oliver A, Winfield IJ (2016) Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology* 25, 3101-3119.

Harper LR, Lawson Handley L, Hahn C, Boonham N, Rees HC, Gough KC, Lewis E, Adams IP, Brotherton P, Phillips S, Hänfling B (2018) Needle in a haystack? A comparison of eDNA metabarcoding and targeted qpcr for detection of the great crested newt (*triturus cristatus*). *Ecology and Evolution* 8, 6330-6341.

Lugg WH, Griffiths J, Rooyen AR, Weeks AR, Tingley R (2018) Optimal survey designs for environmental DNA sampling. *Methods in Ecology and Evolution* 9, 1049-1059.

Majaneva M, Diserud O, Eagle S, Boström E, Hajibabaei M, Ekrem T (2018) Environmental DNA filtration techniques affect recovered biodiversity. *Scientific Reports* 8, 4682-4682.

Muha TP, Robinson CV, Garcia de Leaniz C, Consuegra S (2019) An optimised eDNA protocol for detecting fish in lentic and lotic freshwaters using a small water volume. *PLoS One* 14, e0219218.

R Core Team (2019) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria

Schabacker JC, Amish SJ, Ellis BK, Gardner B, Miller DL, Rutledge EA, Sepulveda AJ, Luikart G (2020) Increased eDNA detection sensitivity using a novel high-volume water sampling method. *Environmental DNA* 2, 244-251.

Smart AS, Weeks AR, Rooyen AR, Moore A, McCarthy MA, Tingley R, Yoccoz N (2016) Assessing the cost-efficiency of environmental DNA sampling. *Methods in Ecology and Evolution* 7, 1291-1298.

Spens J, Evans AR, Halfmaerten D, Knudsen SW, Sengupta ME, Mak SST, Sigsgaard EE, Hellström M (2017) Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: Advantage of enclosed filter. *Methods in Ecology and Evolution* 8, 635-645.

Takahara T, Iwai N, Yasumiba K, Igawa T (2020) Comparison of the detection of 3 endangered frog species by eDNA and acoustic surveys across 3 seasons. *Freshwater Science* 39, 18-27.

Thomas AC, Nguyen PL, Howard J, Goldberg CS (2018) ANDe^{TM}: A fully integrated environmental DNA sampling system. *Methods in Ecology and Evolution*.

Thomas AC, Nguyen PL, Howard J, Goldberg CS (2019) A self-preserving, partially biodegradable eDNA filter. *Methods in Ecology and Evolution*.

Tingley R, Greenlees M, Oertel S, van Rooyen AR, Weeks AR (2019) Environmental DNA sampling as a surveillance tool for cane toad Rhinella marina introductions on offshore islands. *Biological Invasions*, 21(1), 1–6.

Turner CR, Barnes MA, Xu CCY, Jones SE, Jerde CL, Lodge DM (2014) Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* 5, 676-684.

Zhang S, Lu Q, Wang Y, Wang X, Zhao J, Yao M (2020) Assessment of fish communities using environmental DNA: Effect of spatial sampling design in lentic systems of different sizes. *Molecular Ecology Resources* 20, 242-255.