The Importance of Filtration in the Environmental DNA (eDNA) World

Introduction

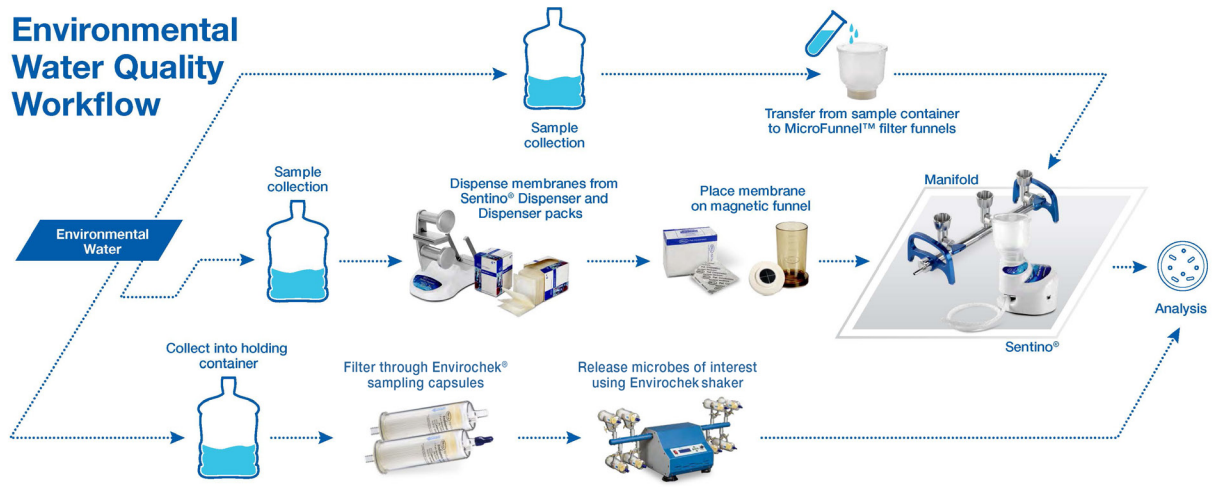
Environmental DNA, or eDNA, is defined in the traditional sense as the mixture of genetic material released from an organism into its environment. Taking advantage of the fact that organisms can shed DNA via skin, hair, scales, feces, or bodily fluids as they move through their aquatic or terrestrial environment[1], eDNA analysis has revolutionized how field biologists detect endangered species. For example, while a particular species of fish may no longer be physically present in an environmental sample taken from a stream or river, the fact that it has migrated through and left traces of genetic material behind allows scientist to detect it. Diluted as such, eDNA is often present in only vanishingly small quantities. It is only in the last decade that novel molecular techniques for detection and analysis have been available. To now be able to trace endangered species and problem invasive species in the wild has led to a spectacular development of eDNA studies. As the field explodes, the definition of eDNA has also evolved, with a recent call from scientists to expand the definition of eDNA to include the genetic material, both intra- and extracellular DNA, of microbial and macrobial species, isolated from an environmental sample[2].

Methods for eDNA Extraction - Filtration versus Precipitation

In general, eDNA analysis involves the following steps: capture, preservation, extraction, amplification, and sequencing to ensure match to target species. Efficiency at each stage has a knock-on effect on the output of subsequent steps, hence the initial steps of capture, preservation, and extraction are especially important as they directly impact the quantity and quality of DNA available for amplification and sequencing. In fact, researchers have found that eDNA recovery varied depending on the protocols or combinations of protocols used and more often than not, researchers prioritized methods based on cost and ease of sampling[3,4]. Because eDNA detection often relies on detecting ultra-low sample concentrations of highly degraded DNA, filtration is typically preferred as it enables the collection of eDNA from large volumes of water or other media. Filtration (the passage of water samples through a filter to trap the DNA) is preferred over precipitation (using ethanol to precipitate nucleic acids in the sample) as the critical capture method step. A study by Hinlo *et al.*, 2017 investigated filtering samples through a 47 mm, 0.8 um cellulose nitrate filter paper using the Pall's Laboratory Manifold and a peristaltic pump in a set-up similar to figure 1 and compared it to a precipitation method and found that the method involving filtration yielded the highest quantities of DNA[5]. This is consistent with other studies that also showed that filtration recovered more eDNA from water samples[3,4,6]. Filtration of water in the field is also logistically more advantageous when dealing with larger bodies of water, compared to transport of water to the nearest lab, and thus, appropriate filter preservation becomes crucial for maximum DNA recovery[7].

Figure 1

Frequently used filtration workflow set-ups in eDNA sampling.



Time of Filtration and Storage

Environmental DNA degrades readily in the environment, and the rate of eDNA degradation increases with higher temperatures and exposure to UV-light[8]. Therefore, it is important to reduce the time between sampling and filtering to retrieve as much eDNA as possible from the sample.

For on-site sampling, many researchers use an electrical vacuum pump connected to a filter holder manifold which allows researchers to combine benefits of both on-site and laboratory filtering, by ensuring optimally fast and sterile filtering conditions are observed during sample collection [Figure 1]. Pall's Sentino® pump and manifold were used in the Nature Research report by Majaneva ***et al.***, 2018[9], where they examined how eDNA filtration techniques affect recovered biodiversity. In their comparative study, the researchers chose to filter the samples at the sampling site and perform immediate filter preservation to minimize time for eDNA decay in the samples[9]. Pall's Sentino pump is ideal for use in the field due to its small footprint and battery operation. Its peristaltic flow design means the sample is pulled through the filter and fluid path, eliminating the need for a vacuum source. It also ensures the fluid flows uniformly in one direction without the potential for back-up and contamination of the sample.

Filtering water at the collection site has clear advantages compared to transporting large volumes of sample water; however appropriate filter preservation is then crucial for maximum eDNA recovery. Best practice dictates that if samples cannot be filtered within 24 hours from collection, refrigeration is necessary at 4 °C. If the samples are to be stored for more than a few days prior to extraction, extracted within a few days, or in case of long-term storage, samples should be placed inside a -20 °C freezer. Time is of the essence. Hinlo ***et al.***, observed a significant decrease in DNA copy numbers from Day 1 to Day 2 in stream samples regardless of storage method[5]. Overall, their study supported the recommendation to filter water samples within 24 hours of collection but if this cannot be done, they suggested short-term refrigeration for up to five days may be a better storage option than freezing[5].

Filtration Material Type and Pore Size

Many factors influence the success of the filtration method. It's important to consider the filter material and pore size in the selected filter, as both can affect the particle retention and flow rate. Glass fiber (GF), cellulose nitrate (CN), mixed cellulose ester (MCE), polycarbonate (PC), nylon, polyethersulfone (PES) and cellulose acetate (CA) are all commonly used filter materials in eDNA studies[10]. It has been postulated that DNA binds to each filter paper differently and in fact this has been supported by research carried out by Liang and Keeley who investigated the effect of filter paper type on the recovery of spiked DNA plasmid and showed that DNA had different binding affinities to different filter papers[11].

Filtration using CN filter paper or MCE filters, which are composed of cellulose nitrate and a small content of cellulose acetate, have been shown in several studies in the eDNA space to be one of the most effective filters that deliver the highest eDNA yield[5,9,12] when compared with other filters such as PES and PC filters. Hinlo ***et al.,*** 2017 postulated that CN and MCE (combination of cellulose nitrate and cellulose acetate) yielded significantly more eDNA than the other filter papers used in eDNA capture because DNA was also trapped within the matrix itself and not only on the surface[5]. While further investigation is underway to determine which factors allow eDNA to bind to some filter types more than others, Pall does supply the full spectrum of filter material types to eDNA researchers.

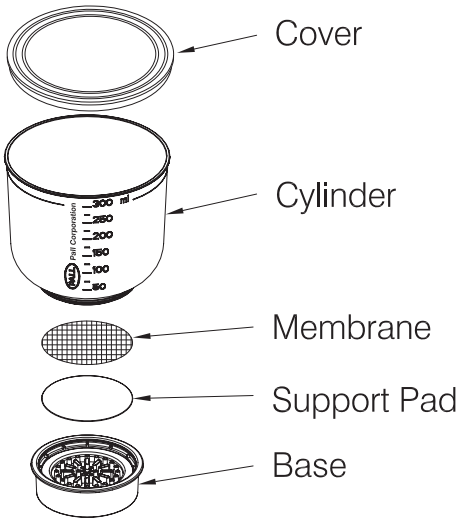
However, eDNA capture from aqueous environments is complicated by factors such as pH, organic and inorganic particles, and filter pore size, all of which are thought to influence the final eDNA yield. In general, small pore size filters yield the most eDNA, but they may clog easily in turbid waters, which results in slow filtering speeds and increases sampling time[6,7,11]. To prevent the latter, either larger pore-size filters or pre-filtration may be used, however pre-filtration can be an expensive and somewhat unnecessary step if methodology is modified. In fact, many researchers prefer to increase the pore size on the filter and Turner ***et al.***, 2014 suggest it is then ideal to increase the target water volume to ensure the yield is equivalent to the eDNA captured using smaller pore-sized filters[7].

Last year, the Alliance for Coastal Technologies in coordination with the U.S. Integrated Ocean Observing System and the U.S. Marine Biodiversity Observation Network held a workshop on the future of eDNA sampling to focus on addressing the challenges and needs during eDNA collection and processing. Here it was reported that the majority of participants relied on filtration to concentrate samples, with filter pore sizes ranging from 0.2 µm - 5 µm[13]. As presented in Pall's overview of the flexible filtration options offered to labs doing eDNA work, their filters easily accommodate this filter pore size range, across many different filter materials. In order to accelerate research in the eDNA space, Pall also works with end users on custom solutions for their needs.

Sterile Conditions, Single-use and Re-use

Sterility is also a concern in the field, as well as ease of use. In fact, when Majaneva ***et al.***, 2018 carried out a comparative study to look at the 2 different material types of filters on eDNA yield, they chose Pall's 0.2 µm PES and Pall's 0.45 µm MCE filters because they are attached to a 300 mL reservoir, sterile and individually packaged making them incredibly easy to use in the field[9] (see Figure 2).

Figure 2



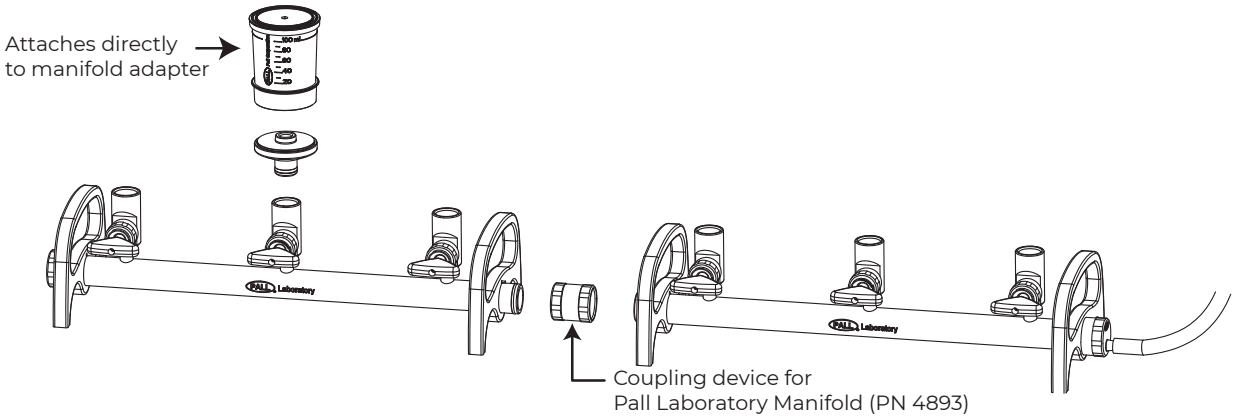
Pall's 300 mL MicroFunnel™ Filter Funnel Overview. Supports a variety of membranes (mixed cellulose ester, polyethersulfone and modified polyethersulfone membrane in 0.2 - 0.8 µm pore sizes.

The location of eDNA sampling can influence workflow process changes in order to simplify the collection and purification of water samples at remote or inconvenient sites, e.g., remote tropical river locations, on boats, etc[14]. Sampling eDNA in tropical systems compared to temperate ecosystems can also be more challenging due to the influence of high water temperature on eDNA shedding and potential degradation. Robson ***et al.***, 2016 made several modifications to their decontamination and filtering eDNA protocols to maximize efficiency when working in tropical systems[14]. To reduce processing and decontamination times, the researchers switched from a magnetic filter funnel to Pall's smaller MicroFunnel (Figure 2), using a 300 mL MicroFunnel with 0.8 µm Metricel® black, gridded membrane (made of hydrophilic modified PES, individually bagged and gamma irradiated) for each sample. According to Robson ***et al.***, "their smaller size and stackable nature make Pall's MicroFunnels ideal for remote fieldwork where space is limited"[14].

Wacker ***et al.***, 2019 and Fossøy ***et al.***, 2020 also chose to carry out eDNA sampling using Pall's 300 mL MicroFunnel[15,16]. In their set up, samples were filtrated using a vacuum pump connected to Pall's Manifold and the filter funnel adaptors. The Pall Manifold allows optimized testing when working in the eDNA field environment without sacrificing cleanliness. The modular design allows two manifolds to combine, yielding a standard 6-place manifold, which can be easily separated for disinfection and/or sterilization (Figure 3).

For the filtration of larger sample sizes (up to 1000 L), the high-capacity Envirochek® HV 1 µm from Pall, with its larger surface area, is ideal as it allows the filtration of much greater volumes of water. Vences ***et al.***, 2016 confirmed its performance in the eDNA space[17]. Researchers also now rely on the Envirochek HV when looking at pathogen detection in water samples. Briefly, in the Envirochek sampling capsule method, the water sample to be tested is passed through the capsule either by sampling at the source or by taking a sample back to the laboratory and filtering it at the bench. The capsule is then filled with an elution solution, placed on a laboratory shaker, and vigorously shaken to elute and capture the microbe of interest. Up to eight capsules can be processed at once using Pall's Envirochek Laboratory Shaker (workflow in Figure 1). The elution solution is decanted and centrifuged to a pellet for further analysis, including nucleic acid extraction.

Figure 3



The modular design of the Pall's 3-place Laboratory Manifold allows two manifolds to combine, yielding a standard 6-place manifold, that can be used in the field to assist multiple samples to undergo simultaneous filtering and can be easily separated for disinfection and/or sterilization.

Conclusion and Future Directions

eDNA has now established itself as one of the main tools in the study of water eco systems. Starting with early roots in simple, low volume, temperate water systems where target organisms are more easily identified, the technology is now employed across a broad range of ecosystems including fresh water and marine environments, and from temperate to tropical environments. Initial studies focused on single target species have been augmented with new techniques targeting multiple species at the genus level and across the entire range of organisms from virus to bacteria, plants, coral, fish, and amphibians.

Researchers continue to push back the boundaries and limitations of the eDNA technology, as well as confirming the accuracy and sensitivity of eDNA by comparison with traditional survey methods. eDNA technology is well established as a tool, particularly within the field of ecology and its breadth of applications will continue to grow rapidly in the foreseeable future. In fact, the emergent science of eDNA is opening up the ability for researchers to also carry out early detection of invasive species and dynamic surveillance of human pathogens. More recently, researchers began repurposing eDNA techniques to detect eRNA in human wastewater. It is these genetic tools that are being employed to better understand and monitor the SARS-CoV-2 virus driving the current global COVID-19 human pandemic[18]. There is an increasing body of evidence that SARS-CoV-2 viral shedding in fecal matter takes place in infected individuals, regardless of whether that individual is symptomatic[19]. Thus, detection of SARS-CoV-2 viral particles in human wastewater provides a sensitive early-warning system for the spread of COVID-19 in communities.

With a full range of filtration devices, filters, funnels, manifolds and the Sentino field-friendly portable pump, Pall continues to support the development of high sensitivity workflows to address the problem of extremely low concentrations of DNA in water samples. Incorporating sterile filtration with expeditated filtration rates and reduced filter clogging into eDNA workflows, ultimately will help the much-needed standardization in the eDNA space.

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