# Optimization of Methods for the Collection of Larval Sea Lamprey Environmental DNA (eDNA) from Great Lakes Tributaries

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## TABLE OF CONTENTS

# ABSTRACT

**Background:** Environmental DNA (eDNA) sampling and analysis have the potential to revolutionize species monitoring, but the effective implementation in field conditions remains uncertain. The current study addresses this knowledge gap by developing a robust eDNA sampling protocol for the detection of larval Sea Lamprey *Petromyzon marinus* in Great Lakes tributaries.

**Methods:** Three experiments were conducted to optimize eDNA field sample collection. The first experiment compared the performance of 0.45-µm, 1.2-µm, 5.0-µm cellulose nitrate (CN) filters and a 1.5-µm glass-fiber filter to determine which filter consistently yielded the highest median DNA copy number. The second experiment evaluated the performance of two filtration devices for eDNA sample collection and filtration, an autosampler (Halltech OSMOS aquatic eDNA sampler, Halltech Environmental and Aquatic Research, Guelph, Ontario, Canada) and a handheld peristaltic pump. In the third experiment, a biweekly eDNA survey was conducted to investigate the temporal dynamics of spawning Sea Lamprey eDNA to determine at what point during the season only larval lamprey eDNA is detected.

**Results:** Our findings indicate that CN filters with a pore size of 1.2 µm or 5.0 µm captured consistently the highest amount of eDNA, but the 5.0-µm CN filter was selected for routine use due to its superior performance and reduced risk of clogging. We found no significant performance differences between the OSMOS aquatic eDNA sampler and the peristaltic pump across three response variables (frequency of contaminated field negative controls, PCR inhibition, and positive detections), suggesting both devices can reliably be used. Moreover, our study found that the spawning Sea Lamprey eDNA signal attenuates approximately 4–6 weeks after the last adult Sea Lamprey capture, which is consistent with previous research.

**Discussion:** By synthesizing the results, we provide a streamlined eDNA sampling protocol for larval Sea Lamprey monitoring. We recommend beginning eDNA sampling at least six weeks after the end of the estimated regional spawning period and using a 5.0-µm CN filter in combination with the OSMOS aquatic eDNA sampler, with the handheld peristaltic pump serving as a backup. This optimized approach improves the efficacy and reliability of eDNA-based monitoring.


# INTRODUCTION

The invasive Sea Lamprey *Petromyzon marinus*, which contributed to the collapse of the Great Lakes fishery (Lawrie 1970), remains a persistent threat to this vital ecosystem and industry (McLaughlin et al. 2021). Since the initial application of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM) in the latter half of the 20th century (Schnick 1972; Smith and Tibbles 1980), TFM has been critical for disrupting the Sea Lamprey life cycle in Great Lakes tributaries by killing larvae prior to their transformation into parasitic juveniles (Wilkie et al. 2019); TFM continues to be used today as the primary means to control Sea Lamprey (Marsden and Siefkes 2019; Wilkie et al. 2019). Electrofishing serves as the main method for assessing larval Sea Lamprey presence, abundance,

and size distribution (Slade et al. 2003). These data, when inputted into the Empiric Stream Treatment Ranking system (Christie et al. 2003; Hansen and Jones 2008), guide the selection of tributaries with the most favorable cost-to-kill ratio for TFM treatment. In the historical record, more than 500 out of 5,311 (9.4%) Great Lakes tributaries have faced Sea Lamprey infestations (Barber and Steeves 2020). Yet, constraints in the Great Lakes Fishery Commission's (GLFC) Sea Lamprey Control Program's budget and staffing allow for treatment of only one quarter of infested streams each year (Jubar et al. 2021). Also, electrofishing surveys have limitations. Steeves et al. (2003) demonstrated that, even at medium to high larval Sea Lamprey densities, the probability of detection through electrofishing was only 0.48; in cases of low larval Sea Lamprey densities, electrofishing performance was even less effective. Additionally, electrofishing cannot usually be conducted from late October to May nor in areas with water too deep to wade for backpack electrofishing or impassable for boat-based electrofishing. Given the number and extent of tributaries in the Great Lakes basin, an exploration of alternative methods to supplement electrofishing could greatly enhance Sea Lamprey surveillance.

Amid these challenges, the emergence of environmental DNA (eDNA) monitoring presents a possible supplement to electrofishing as a method of detecting larval Sea Lamprey infestations. Research has shown that for detecting low-density aquatic macro-organisms, eDNA can be more effective than electrofishing (Sigsgaard et al. 2015; McKelvey et al. 2016; Wilcox et al. 2016, 2018; Strickland and Roberts 2019), and it is a promising avenue for furthering Sea Lamprey control measures (Docker and Hume 2019). In addition, eDNA methods have been used to detect species in winter (Feng et al. 2020; Khalsa et al. 2020), which would extend the monitoring season for larval assessment. Thus, although follow-up electrofishing surveys would be required to determine abundance and larval size distribution, eDNA screening across large spatial scales could permit efficient detection of new Sea Lamprey infestations, monitor for reinfestation after TFM treatment, determine instream distribution prior to lampricide treatment, and extend the field season. A first pass using eDNA monitoring could permit more efficient deployment of electrofishing crews.

Those in ecosystem management roles may find the burgeoning use of eDNA for monitoring aquatic species of management and conservation concern encouraging, prompting them to consider adopting eDNA methods for monitoring their species of interest. However, a multitude of eDNA methods are used to monitor aquatic macro-organisms (Tsuji et al. 2019), with many of these specialized methodologies being developed independently by different research groups (Goldberg et al. 2016). This proliferation of techniques, while exciting on one hand, has also complicated the selection of eDNA methods for novel applications, hindering the reproducibility of studies and adoption of eDNA methods for species detection (Tsuji et al. 2019). Ecosystem managers may face uncertainty when deciding which eDNA methods to use, as the methods used can affect the efficacy of eDNA collection and processing (Hinlo et al. 2017).

The extensive use of electrofishing in the GLFC's Sea Lamprey Control Program (Hansen and Jones 2008) presents an opportunity to evaluate the use of eDNA techniques for detecting larval Sea Lamprey. In previous studies (Gingera et al. 2016; Schloesser et al. 2018), using polymerase chain reaction (PCR) assays to detect Sea Lamprey eDNA has shown promise, both within controlled-density tanks and in Great Lakes tributaries. However, it is crucial to note that, prior to the present study, an optimized and thoroughly validated eDNA sampling protocol specific to larval Sea Lamprey within Great Lakes tributaries did not exist. This gap in knowledge is crucial because, without an optimized protocol, those in ecosystem management roles may hesitate to adopt eDNA methods (Loeza-Quintana et al. 2020).

The process of using targeted eDNA methods for aquatic species encompasses six primary stages: (1) collecting water samples, (2) filtering water samples, (3) preserving the eDNA and other non-aqueous materials on the filter, (4) isolating eDNA, (5) quantitative (qPCR) analysis, and (6) statistical analysis and data interpretation (Langlois et al. 2021). Steps 1–3 are typically conducted in the field (steps 1 and 2 are combined when using an automated eDNA sampler), and steps 4–6 are performed in the laboratory. Furthermore, the ecology of the target species should guide decisions on where and when to collect eDNA samples (Rees et al. 2014). By comparing different sample collection and filtration methods and analyzing the impact of the target species' ecology on eDNA detection, a unified sampling protocol can be developed for future efforts.

There are an almost infinite number of combinations of different parameters that have been used in eDNA studies, and the diverse applications of eDNA do not lend themselves to a one-size-fits-all protocol (Kumar et al. 2020). However, we identified parameters that were already deemed to be effective based on our previous experience (e.g., Gingera et al. 2016; Schloesser et al. 2018; Loeza-Quintana et al. 2020, 2021; Milián-García et al. 2021) and review of the literature versus parameters that required more thorough testing and optimization for our specific needs. For example, best practices dictate that water samples be filtered immediately on-site rather than transported back to the laboratory (Laramie et al. 2015). On-site water filtration systems used for eDNA typically include portable peristaltic pumps, automated eDNA samplers, and vacuum pumps with a filter funnel manifold. Because a vacuum pump would require access to a less portable power source (e.g., a streamside generator), we tested only the first two options. Similarly, although eDNA preservation can be achieved by storage in 95–100% ethanol, RNAlater, or Longmire's or ATL buffer, (Majaneva et al. 2018; Kumar et al. 2020), handling large volumes of ethanol or other liquid storage media (and needing to keep them on ice or at -20°C) is not practical for Sea Lamprey larval assessment teams, which are often away from laboratory or other sample storage locations for more than one week. Majaneva et al. (2018) concluded that silica gel desiccant improved species detection and resulted in lower variability relative to other filter storage media. Also, during subsequent processing, dried filters are easily broken into pieces in microcentrifuge tubes, reducing handling time and permitting sub-sampling (e.g., for archiving or method comparison).

Researchers should aim to maximize eDNA capture from water samples, and the choice of filter type is significant due to eDNA's varied forms in water. Smaller pores capture intracellular and smaller extracellular DNA fragments, but they can more easily be clogged by particles like clay and soil (Kumar et al. 2020). Conversely, filters with larger pores work well for capturing larger eDNA fragments and clog less than smaller-pored filters, but they may allow smaller fragments to pass through the filter (Turner et al. 2014). However, small filter pore sizes, as highlighted by Kumar et al. (2020), may pose a risk. Even filters with relatively large pores, such as 1.5 µm, can become clogged in turbid environments. This clogging can lead to inhibition and reduced sample volumes, potentially hampering detection. Thomas et al. (2018) recommended a 5.0-µm pore size due to its lower risk of clogging and higher DNA yield when filtering larger volumes. Alongside pore size, filter material is another factor to consider. In their guidance document, Vazquez et al. (2023) highlight that filter materials such as cellulose nitrate (CN), glass fiber (GF), and mixed cellulose ester are commonly employed in aquatic eDNA studies. Use of these materials is supported by findings in the research of Majaneva et al. (2018) and Muha et al. (2019). In prior research, 1.5-µm GF filters were used to collect larval Sea Lamprey eDNA (Gingera et al. 2016; Schloesser et al. 2018). However, a study by Hinlo et al. (2017) comparing filter types for a different aquatic invader, the Oriental Weatherloach *Misgurnus anguillicaudatus*, found that, when using the DNeasy® Blood & Tissue kit (Qiagen, Venlo, The Netherlands) for extractions, 1.2-µm GF filters had a significantly lower DNA yield compared to 1.2-µm CN filters.

Laramie et al. (2015) and Nolan et al. (2023) highlight the significant impact the choice of water-sample filtration device can have on the effectiveness of eDNA studies. To establish an eDNA field methods protocol that ensures the production of dependable species-occurrence data, researchers should thoroughly compare filtration devices commonly used in eDNA research. This assessment involves evaluating various critical factors, including the potential for contaminated negative controls (resulting in false positives), the risk of PCR inhibition and the likelihood of missing the target organisms (leading to false negatives), and the effectiveness of detecting the target organism(s).

Determining the appropriate time window for sampling is a crucial aspect of an eDNA field methods protocol. The Sea Lamprey's life history complicates eDNA monitoring, as PCR analysis cannot differentiate between adult/spawner and larval Sea Lamprey eDNA, both of which occur within tributaries. In spring, adult Sea Lamprey in the Great Lakes migrate upstream to spawn (Manion and Hansen 1980), often guided by pheromones released by larval Sea Lamprey (Vrieze et al. 2010). After spawning, adult Sea Lamprey die, while the larvae remain burrowed in the sediment for typically 3–5 years until they metamorphose into parasitic juveniles and out-migrate to the lakes (Dawson et al. 2015). To accurately monitor larval Sea Lamprey without conflating detections with spawning adults or their decomposing remains, sampling must take place after spawner eDNA has dissipated from the tributary. Gingera et al. (2016) used conventional PCR (cPCR) to establish that the eDNA signal from spawners in the Little Thessalon River of northern Lake Huron diminished around July 22, approximately one month after the end of the spawning run. However, given the higher sensitivity of qPCR compared to cPCR (Xia et al. 2018), it is possible that spawner eDNA will be detected longer with qPCR;

therefore, it is important to test spawner eDNA attenuation times with qPCR to determine the time after which eDNA detected in a stream can be assumed to originate exclusively from larvae. Additionally, as summarized by Rourke et al. (2022), a multitude of biotic factors influence eDNA concentrations and dispersion in aquatic systems. For example, the flow rate and hydrology of specific river systems affect eDNA dispersion (Harrison et al. 2019; Rourke et al. 2022) and influence how long eDNA remains in the aquatic environment after the target species has departed.

To test and refine eDNA field sampling methods for larval Sea Lamprey, we conducted three experiments. These methods were based on the species' ecology, and they were designed to be efficient and sensitive to its presence, while minimizing the risk of both false-positive and false-negative errors. The first experiment assessed various filter types to determine the most suitable material and pore size for our research scenario. In the second experiment, we compared two filtration devices: the OSMOS Aquatic eDNA sampler (Halltech Environmental and Aquatic Research, Guelph, Ontario, Canada; Nolan et al. 2023) and a handheld do-it-yourself peristaltic pump described in Gingera et al. (2016). In the third experiment, we conducted biweekly eDNA surveys in a Great Lakes tributary during July and August to determine when eDNA detections of adult Sea Lamprey stop after the spawning run. By synthesizing the results of our research, we aim to develop an efficient and sensitive eDNA sampling protocol for detecting the presence of larval Sea Lamprey. Our goal is to provide an effective supplement to electrofishing to enhance the surveillance of larval Sea Lamprey in Great Lakes tributaries.

# METHODS

## Experiment 1: Assessing Filter Material and Pore Size

### Study Design and Site Selection

This experiment's objective was to identify the filter type that consistently collected the highest quantity of eDNA. At each location, one field negative control and four biological samples per filter type were collected and filtered; the negative control tested for possible contamination in the equipment or during collection and filtration. A total of 2 L of tap water was filtered for each negative control, with 10 L and 2 L of river water filtered for each biological sample in the first and second sampling activities, respectively, using the OSMOS aquatic eDNA sampler. Samples for each filter type, starting with the negative control, were consecutively filtered. As per the standard protocol, if a filter clogged before reaching the desired water volume, the filter was preserved, and the clogging event and filtered volume were noted. Filtration volume was decreased from 10 L to 2 L in the second experiment, because 2 L was the largest volume that could be consistently filtered at most locations. The field data sheet (see Supplementary Information S1: Environmental DNA Sampling Field Data Sheet in the online Supplement of this publication) used to record sampling metadata was a modified version of the GEN-FISH Lotic eDNA Collection Data Sheet (GEN-FISH 2022a). Upon returning from field collection, filters were kept at −20°C until DNA extraction occurred. Sterile techniques were used for the collection of all samples to minimize the risk of contamination that could lead to false positives. Briefly, these techniques included

thorough decontamination of all non-single-use sampling supplies. For materials that came into direct contact with the samples (e.g., gloves, forceps, storage bags, filter canisters, tubing), items were either changed between sampling stations or thoroughly decontaminated to prevent cross-contamination between sampling stations. For the complete guide on eDNA sample collection, see Appendix.

We conducted two independent field trials to determine the optimal filter type only at locations that contained larval Sea Lamprey densities greater than 0.1 larvae/m$^2$. Low target-organism density can cause stochasticity in the quantity of eDNA captured across field sample replicates (Van Driessche et al. 2023); thus, to reduce the effect of this stochasticity, we restricted sampling to areas with relatively high larval densities (Sea Lamprey Control Centre (SLCC)), Fisheries and Oceans Canada (DFO), unpublished data). The first field trial took place in September 2021, at a sampling station on Big Otter Creek (42.854 405° N, -80.724 185° W) in the municipality of Tillsonburg, Ontario. The second field trial occurred in June 2022, at a sampling station on the Credit River (43.632 731 5° N, -79.759 016 8° W) in the municipality of Brampton, Ontario (Figure 1). In 2020, electrofishing surveys at the Big Otter Creek station reported an average larval density of 3.3 larvae/m$^2$, and, in 2021, electrofishing surveys at the Credit River station reported an average larval density of 0.5 larvae/m$^2$ (L. Sumner, DFO; unpublished data).

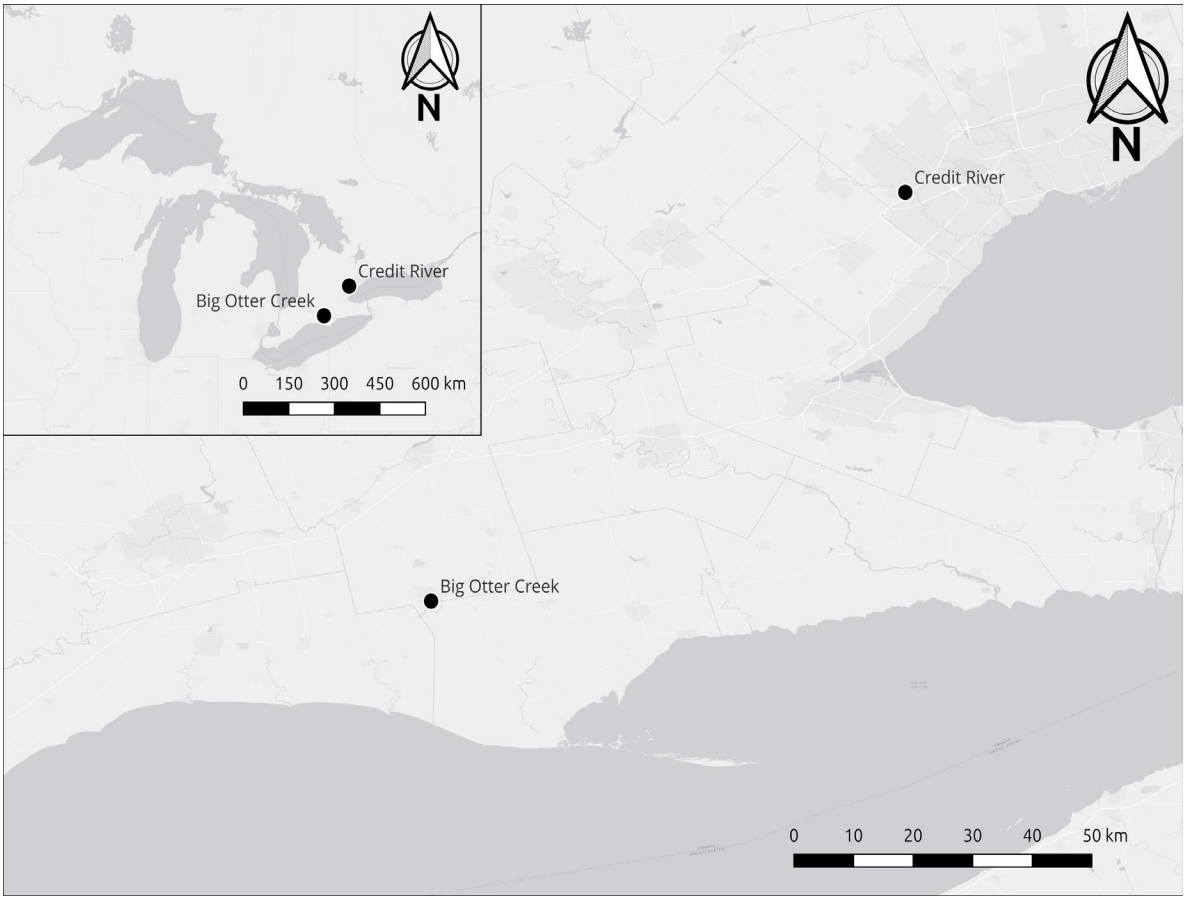


FIGURE 1. Locations of Big Otter Creek and Credit River, Ontario, where eDNA sampling was conducted for the filter comparison experiment (Experiment 1). At each sampling location, four biological replicates and one field negative control were collected for each of the four filter types. Map was created using QGIS Desktop (available at https://qgis.org/).

**Filter Selection**

The four filter types selected for the experiment were 1.5-µm GF, and 0.45-µm, 1.2-µm, and 5-µm CN filters. The 1.5-µm GF filters were included based on their previous use in eDNA monitoring of Sea Lamprey (Gingera et al. 2016; Schloesser et al. 2018). Additionally, CN filters were tested, as previous research suggested a positive interaction between CN material and the DNeasy® Blood & Tissue Kit (Qiagen, Hinlo et al. 2017) used for DNA extractions. The three pore sizes for CN filters were chosen to assess the potential influence of filter pore size on DNA yield. Multiple pore sizes for GF filters were not included due to limited availability from manufacturers.

**Extraction and qPCR Analysis**

DNA extraction from the collected filters was conducted within four months of the sample collection. DNA extraction followed a modified manufacturer's protocol (see Supplementary Information S2: DNA Extraction Protocol One in the online Supplement Supplement of this publication) using a DNeasy® Blood & Tissue Kit (Qiagen) and a QIAshredder (Qiagen). Each batch of extractions included a new 1.5-µm GF filter as an extraction negative control to test for contamination during this step. The DNA extracts were stored at -20°C until needed for qPCR analysis. To quantify Sea Lamprey DNA in each sample, a TaqMan™ Assay (Thermo Fisher Scientific, Waltham, Massachusetts) was used (Table 1). The assay targeted a 154-base pair segment of the Cytochrome B (CytB) gene, as described by Schloesser et al. (2018), which was duplexed with an internal positive control (IPC, TaqMan™). The IPC consisted of synthetic DNA, along with primers and a probe, which were included in the qPCR reaction. This control served to differentiate genuine negative detections from false negatives caused by PCR inhibitors (Gasparini et al. 2020). The qPCR reactions also included a PCR inhibitor-resistant Environmental Master Mix (EMM 2.0, TaqMan™) to further mitigate the impact of inhibitors. The cycling conditions of the duplexed qPCR assay were a 10-minute hold at 95°C, followed by 45 cycles of 15 seconds at 95°C, and 60 seconds at 60°C.

Table 1. Sea Lamprey qPCR assay details. Forward primer, reverse primer, and probe (with a (Fluorescein (FAM) dye label) for the TaqMan™ qPCR assay targeting the Cytochrome B (CytB) gene of the Sea Lamprey and the sequence of the resulting 154-base pair amplicon. In the probe sequence, ZEN™ is the internal quencher (Integrated DNA Technologies, Coralville, Iowa). From Schloesser et al. (2018).

| Component | Sequence |
|---|---|
| Forward primer | 5'-GCTTCTGTAATCTACCGGCAT-3' |
| Reverse primer | 5'-GTAGAAATGGCATAGGCAAATAGA-3' |
| Probe | 5'-TTCCCTTTT/ ZEN/AGCCCTAATGCACT-3' |
| 154-base pair gene fragment CytB | 5'-TGGT TTT GTT ATT CTA CTG GGC ATT CTT TTC ATA ATT TCC TATA GCC CCT AAT GCA CTA GGT GAA CCA GAC AAC TTT ATT GGA AAT CCT CTT AGT ACC CCT CCC CAT ATT AAA CCA GAA TAC TTT CTA TTT GCC TAT GGC ATT CTA C-3' |

The samples collected from the Big Otter Creek sampling station were analyzed on the QuantStudio™ 7 real-time PCR system (Thermo Fisher Scientific), with four qPCR technical replicates per sample. The samples collected from the Credit River sampling station were analyzed on the Mic real time PCR system (Bio Molecular Systems, Dural, New South Wales, Australia), with eight qPCR technical replicates per sample. This doubling of the qPCR replicates for the second set of samples aimed to investigate if heightened sensitivity, achieved through more qPCR replicates (Klymus et al. 2020), would significantly alter the experimental results. Additionally, using eight technical replicates aligns closely with the intended field application methods. The change of qPCR platform from the QuantStudio™ 7 for samples from Big Otter Creek to the Mic for samples from

the Credit River location was made for practical reasons, as the QuantStudio™ 7 was experiencing technical issues at the time. However, the same assay, reagents, and cycling conditions were used for both locations and, most importantly, the data were not statistically compared between the two platforms. Although rigorous comparisons between platforms would be needed before both were used interchangeably during routine eDNA monitoring. The change in platforms here should not impact the interpretation of the results. During each analysis, qPCR technical replicates for each sample were evenly distributed across four qPCR runs. This distribution captured the inter-run variation within individual sample variances. Additionally, each qPCR run included three no-template controls, which served as PCR negative controls to test for contamination at this step. For samples from the first location (Big Otter Creek), a no-amplification control (TaqMan™) was incorporated into each qPCR run. The no-amplification control inhibited the enzyme activity required for PCR reactions and controlled for any non-PCR fluorescence. Additional details about the qPCR assay can be found in Supplementary Information S3: qPCR Assay Details in the online Supplement of this publication.

**Data Analysis**

The average estimated DNA copies/µL of the DNA template for each qPCR reaction was calculated following the method outlined in Klymus et al. (2020). The dilution series used to estimate DNA copies/µL, and establish the limit of quantification (LOQ) and limit of detection (LOD) consisted of a six-fold 1:5 dilution series of synthetic Sea Lamprey DNA gBlock™ from Schloesser et al. (2018), with 15 technical replicates per dilution, which was analyzed on the QuantStudio™ 7. The LOQ was determined to be 24 DNA copies/µL, and the LOD for four and eight technical replicates per sample was 1.858 DNA copies/µL and 1.068 DNA copies/µL, respectively (see Supplementary Information S3: qPCR Assay Details available in the online Supplement of this publication). The standard curve used to assess the efficiency of the assay consisted of a five-fold 1:10 dilution series of synthetic Sea Lamprey DNA gBlock™ from Schloesser et al. (2018), run on the QuantStudio™ 7 with eight technical replicates per dilution, with an efficiency of 96.58% and an $R^2$ of 0.9842 (see Supplementary Information S3: qPCR Assay Details available in the online Supplement of this publication). The synthetic Sea Lamprey DNA gBlock™ sequence allows for a known quantity of DNA to be aliquoted into a qPCR reaction to serve as a positive control to determine qPCR run quality and to also establish performance metrics such as sensitivity, and the conversion of an unknown sample to an estimated average DNA copy number using a standard curve.

In our study, a number of qPCR replicates amplified below the established LOQ, necessitating a method to handle these values statistically. Klymus et al. (2020) note that, in analytical chemistry, it is common to assign qualitative or semi-quantitative values to data falling between the LOD and LOQ. However, in the field of eDNA), there is limited precedent for managing qPCR data points in this range. A frequent approach in various analytical chemistry methods involves using half-LOQ or midpoint values for all amplifications below the LOQ when conducting statistical analyses (Warth et al. 2012; Abia et al. 2013; Li et al. 2018; EMA 2022). Other common methods include excluding the data points, using a likelihood estimation to impute values, and including the values as is (Keizer et al. 2016). Of these methods, the half-LOQ method is suitable when only a

small portion of the data falls below the LOQ. However, when a larger portion of the data set includes values below the LOQ, using those values without adjustment introduces the least bias into the analysis (Keizer et al. 2016). As such, the copy number values below the LOQ were included as estimated in the subsequent statistical analysis and reactions without a machine-registered quantification cycle (Cq) value were assigned a DNA copy number of 0 DNA copies/µL. While this decision to include all values as is may introduce bias into the statistical analysis, it is likely preferable to the other approaches.

Normal quantile-quantile plots were used to visually assess the distribution of estimated DNA copies/µL values grouped by filter type, followed by a Shapiro-Wilk test of whether the assumption of normality had been violated within each group. For samples from both locations, the assumption of normality was violated as evidenced by the skew in the quantile-quantile plots, particularly at the Credit River location (Figure 2). In addition, only the average eDNA counts for the CN5 filters from Big Otter Creek were not statistically significant and did not violate normality (Table 2). Given the non-normal distribution of the data, the non-parametric Kruskal-Wallis test was used to evaluate if there were significant differences in the median DNA copy number among the four filter types. If the Kruskal-Wallis test results were significant ($P < 0.05$), a Dunn's multiple comparison test was used as a post hoc test. This test was used to identify the filter type with the highest median DNA copies/µL, indicating its suitability for future applications. Samples from each location were analyzed separately. Additionally, values for the 1.5-µm GF filter from the Big Otter Creek samples were excluded from the analysis because the field negative control for those samples was contaminated, rendering any subsequent results from those samples untrustworthy.

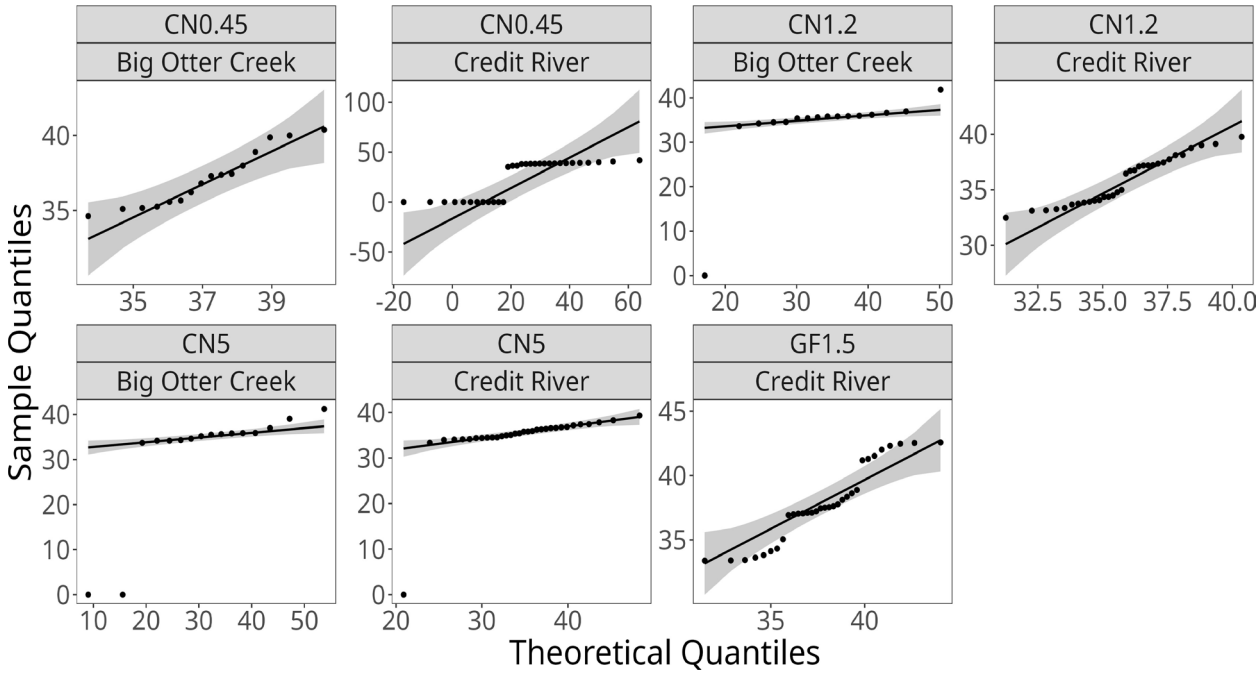


FIGURE 2. Normal quantile-quantile plots for the estimated average DNA copy number for each qPCR replicate from biological samples collected from the Big Otter Creek and Credit River sampling stations in Experiment 1. The plots are a visual check to see if the data are normally distributed; the more closely the data fit the central line, the more closely they approximate a normal distribution. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm.

Table 2. dProbability values estimated with the Shapiro-Wilk test to assess normality of DNA copy number for each filter type for Experiment 1 on the Big Otter Creek and Credit River, Ontario, locations. If the *P*-value was significant at the 5% level, the normality assumption was considered violated and is indicated with an asterisk. For the filter-type abbreviations, the first two characters represent the filter material, and the numbers represent the filter pore size in µm.

| Filter type | Big Otter creek | Credit River |
|---|---|---|
| CN0.45 | $4.10 \times 10^{-2}$* | $1.05 \times 10^{-8}$* |
| CN1.2 | $2.89 \times 10^{-2}$* | $1.75 \times 10^{-4}$* |
| GF1.5 | | $1.76 \times 10^{-7}$* |
| CN5 | $8.19 \times 10^{-2}$ | $3.64 \times 10^{-3}$* |

# Experiment 2: Comparing Two Filtration Devices for eDNA Sample Collection

## Study Design and Location

Experiment 2 aimed to compare two water filtration devices for eDNA sampling. The experiment involved paired eDNA sampling for larval Sea Lamprey at 28 sampling locations. The comparison was based on three key criteria: (1) the frequency of contaminated field negative controls, (2) the frequency of PCR inhibition in field samples, and (3) the frequency of samples containing Sea Lamprey DNA.

In collaboration with the U.S. Fish and Wildlife Service (USFWS) and DFO Sea Lamprey larval assessment teams, paired peristaltic pump and OSMOS sampling occurred at 28 stations across nine tributaries in the USA and Canada during the fall of 2021 (Figure 3). Though other autosamplers exist, the OSMOS was selected due to the flexibility and cost effectiveness of allowing us to use our own filters. At each station, four samples were collected using the OSMOS aquatic eDNA sampler and the peristaltic pump, consisting of one negative control collected from a bucket of tap water, as described in the previous section, followed by three biological replicates. For each sample, 2 L of water was filtered through a 1.5-µm GF filter; however, approximately 10% of the filters clogged before reaching the 2 L mark. The 1.5-µm GF fiber filters were chosen due to their prior use in Great Lakes Sea Lamprey eDNA sampling (Gingera et al. 2016; Schloesser et al. 2018) and because the results of the filter optimization experiment were not yet available. After water filtration, each filter was placed into a uniquely labeled coin envelope and preserved with 100 g of desiccated silica. The coin envelope with the field negative control was stored in its own resealable plastic bag, separate from the three coin envelopes containing filters from the biological replicates (see Appendix). In total, 232 water samples were collected, 116 with the peristaltic pump and 116 with the OSMOS aquatic eDNA sampler.

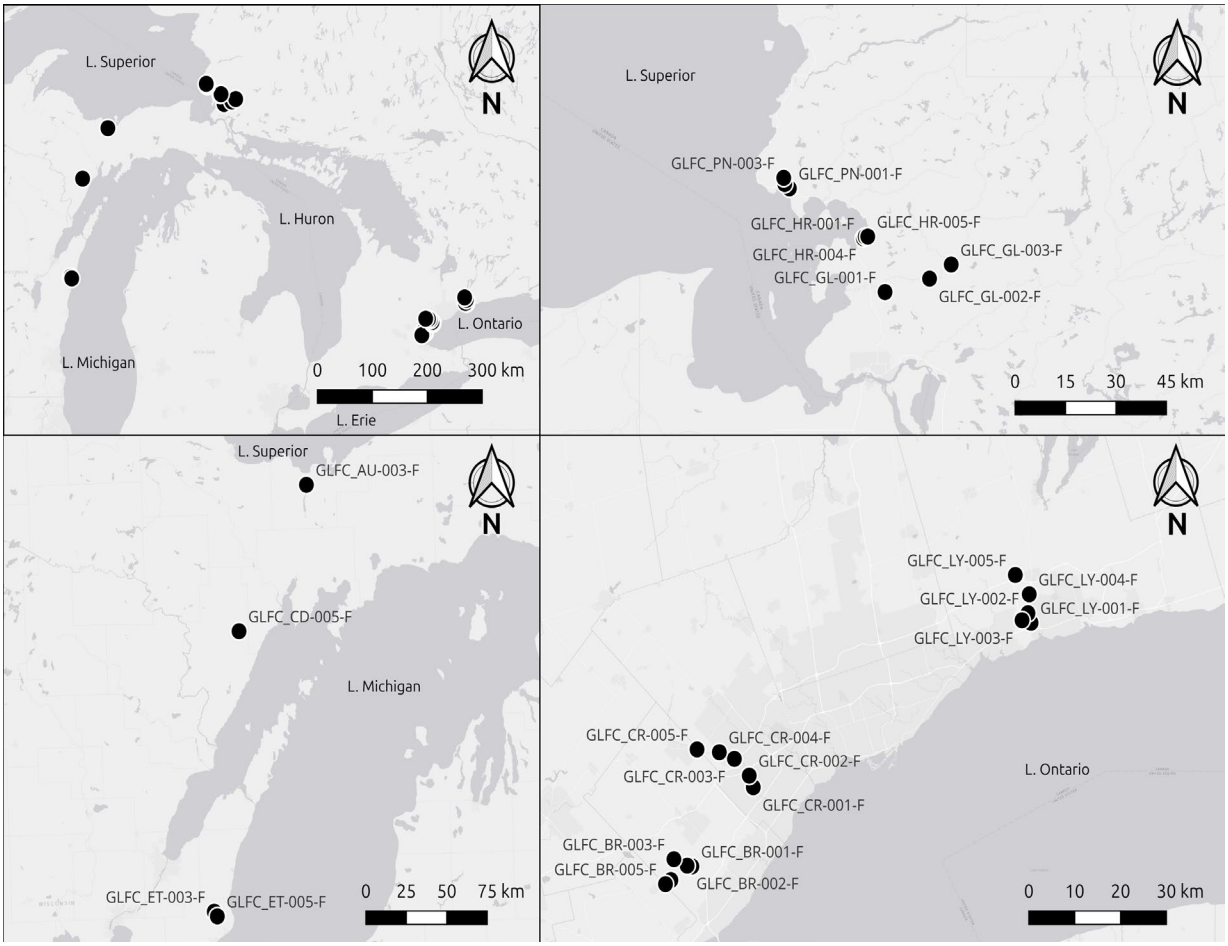


FIGURE 3. Map of the 28 stations sampled for Experiment 2. At each sampling station, three biological replicates and one field negative control were collected with an automated OSMOS aquatic eDNA sampler (Halltech OSMOS) and a do-it-yourself peristaltic pump, described in Gingera et al. (2016). Samples were then analyzed to compare each filtration device with respect to the frequency of detection, contamination of the field negative control, and prevalence of PCR inhibition. Map was created using QGIS Desktop (available at https://qgis.org/).

As in Experiment 1, the field data sheet (see Supplementary Information S1: Environmental DNA Sampling Field Data Sheet available in the online Supplement of this publication) used to record sampling metadata was a modified version of the GEN-FISH Lotic eDNACollection Data Sheet (GEN-FISH 2022a). For the coordinates of the 28 sampling stations, see Supplementary Information S4 (Sampling Station Coordinates for the Filtration Device Comparison available in the online Supplement of this publication).

**DNA Extraction and qPCR Analysis**

The eDNA sample extraction and analysis followed the same methods as Experiment 1, with the following modifications: in the winter of 2022, the analysis was conducted using 96-well qPCR plates on the QuantStudio™ 7, with four qPCR technical replicates per sample. Additionally, each 96-well qPCR plate included a dilution series of Sea Lamprey DNA gBlock™ (with concentrations of 724, 7240, and 72,400 DNA copies/µL) as a positive control, four no-template controls, and one no-amplification control.

**Data Analysis**

The fractional number of cycles in a PCR reaction required for fluorescence to reach a quantification threshold is called the quantification cycle (Cq). The reaction conditions for the qPCR analysis used 45 cycles (see Supplementary Information S3: qPCR Assay Details available in the online Supplement of this publication), so the potential range of Cq values was from 0 to 45. Each sample was classified as inhibited or uninhibited by averaging the Cq value for the IPC across the four qPCR technical replicates. A threshold for inhibition was established by averaging the Cq values from the no-template controls (27 Cq) and extending above this value by 3 Cq (Hartman et al. 2005), corresponding to approximately a one order of magnitude lower concentration than expected. This reduction in concentration is well beyond the expected qPCR error variance and signifies a significant decrease in assay sensitivity due to inhibition (Hartman et al. 2005). Biological samples with an average IPC Cq value below 30 were classified as uninhibited (0), and values above this threshold were classified as inhibited (1). The inhibition outcome (0,1) for the eDNA samples was compared with the filtration device as the treatment level.

To test the effect of the two filtration devices on each of the three response variables, a generalized linear mixed model (GLMM) fitted by maximum likelihood (Laplace approximation) with a binomial error distribution was implemented in R Statistical Software (v4.3.1; R Core Team 2023) using the lme4 package (version 1.1-35.5; Bates et al. 2015). This model allowed exploration of the filtration device effect on the likelihood of sample inhibition. The GLMM was structured with the response variable representing the proportion of inhibited samples. The instrument used was introduced as the fixed effect, while random effects were incorporated to address variations at the river level.

Similarly, an analysis implementing a GLMM, as described above, was used to investigate potential differences in the proportion of contaminated field negative controls between the two filtration devices. The GLMM was structured with the response variable representing the proportion of contaminated field negative controls collected by each

filtration device. Each negative control sample collected was classified as contaminated (1) or non-contaminated (0), with any amplification of the CytB assay in the four technical replicates classified as contamination. Following this, stations where inhibited or contaminated eDNA samples were collected by either filtration device were removed from the data set.

The last analysis focused on evaluating potential differences in the proportion of detected samples between the two filtration devices. The GLMM was structured with the response variable representing the proportion of detected samples, with the filtration device as the fixed effect. Detection was classified as positive if any of the four qPCR replicates from the field samples amplified.

## Experiment 3: Determining the Attenuation of Adult Sea Lamprey eDNA Signal in a Great Lakes Tributary

### Study Location

River selection was crucial for experiment accuracy. The chosen river needed a reliable history of large spawning runs and minimal to no larval Sea Lamprey presence. This choice was driven by the need to accurately determine when the eDNA from spawning Sea Lamprey had dissipated from the system, without ambiguity caused by the presence of eDNA from larval Sea Lamprey. The Humber River (Ontario) has a large spawning run of Sea Lamprey in the spring up to a series of six Sea Lamprey barriers in the 1-km reach upstream of our sampling site, with the first barrier being approximately 150-m upstream of our sampling location (Sea Lamprey Control Map; http://data.glfc.org). Furthermore, despite evidence of egg survival to the pro-larval stage, no larval Sea Lamprey have been detected in recent years by electrofishing or Bayluscide surveys (R. Booth, DFO, personal correspondence). Bayluscide (Bayer 73) is a toxin sprayed on top of the water that causes larval Sea Lampreys to die and rise to the water's surface; it is used both for population control and abundance estimations (Howell et al. 1964). Thus, since larvae are not present in the Humber River, the eDNA signal was expected to drop to zero when spawner eDNA had left the system.

### eDNA Sample Collection

The eDNA sampling took place at five time points on the Humber River (43.652 276 6° N, −79.492 065 7° W) beginning on July 2, 2022, with each sampling activity spaced two weeks apart. During each eDNA sampling activity, a field negative control was filtered from a sterile bucket of tap water, followed by the collection of three biological replicates from the river, using the OSMOS aquatic eDNA sampler in conjunction with 5.0-µm CN filters (Cytivia), a choice informed by the results of Experiment 1. Filtration proceeded until either 2 L of water had been filtered or the filter became clogged. Out of the 15 field samples collected, one filter became clogged, filtering only 0.5 L of water instead of the intended 2 L. Following sample collection, each filter was placed into a uniquely labeled coin envelope and then preserved in 100 g of desiccated silica in resealable plastic bags. After returning from the field, the filters, still preserved within the coin envelopes and desiccated silica, were stored at -20°C to await DNA extraction. The

same field data sheet used to record sampling metadata in Experiments 1 and 2 was used for this survey (see Supplementary Information S1: Environmental DNA Sampling Field Data Sheet available in the online Supplement of this publication; GEN-FISH 2022a). See Appendix for the procedure for eDNA sample collection.

### DNA Extraction and qPCR Analysis

DNA extraction from the filters occurred 2–4 months after collection. DNA extraction from the filters was performed using a Qiagen DNeasy® Blood & Tissue Kit and QIAshredder, following a modified version of the manufacturer's protocol (see Supplementary Information S2: DNA Extraction Protocol Two available in the online Supplement of this publication). Each extraction batch included an unused 5.0-µm CN filter as an extraction negative control to check for lab-based contamination. Extracts were stored at -20°C until qPCR analysis. All samples underwent qPCR analysis with eight technical replicates using the duplexed assay described in Experiment 1. Each qPCR run also included a positive control (7240 DNA copies/µL), six no-template controls, and a no-amplification control.

### Data Archiving

For all three experiments, field data sheets, photographs, lab data, and qPCR RDML files were archived in the Hanner Lab information system (Borisenko et al. 2024).

# RESULTS

## Experiment 1: Assessing Filter Material and Pore Size

The Kruskal-Wallis test indicated significant differences in median DNA copy numbers among filter types for the eDNA samples from the Credit River ($P = 7.631 \times 10^{-10}$), but not for samples from Big Otter Creek ($P = 3.449 \times 10^{-1}$), at $\alpha = 0.05$. As the Kruskal-Wallis test was not significant for the samples from the Big Otter Creek location (Figure 4), a Dunn's multiple comparison test was not applied. At the Credit River sampling station, the median DNA copies/µL was significantly higher for the 1.5-µm GF filter and the 1.2-µm and 5.0-µm CN filters compared to the 0.45-µm CN filters (Table 3, Figure 5). Notably, no significant difference in median DNA copies/µL was observed between the 1.2-µm CN and 5.0-µm CN filters in either location (Table 3; Figures 4 and 5). In the analysis of samples from Big Otter Creek, no fluorescence was observed in the no-amplification controls. This observation led to the decision to not include no-amplification controls for the qPCR runs for samples from the Credit River.

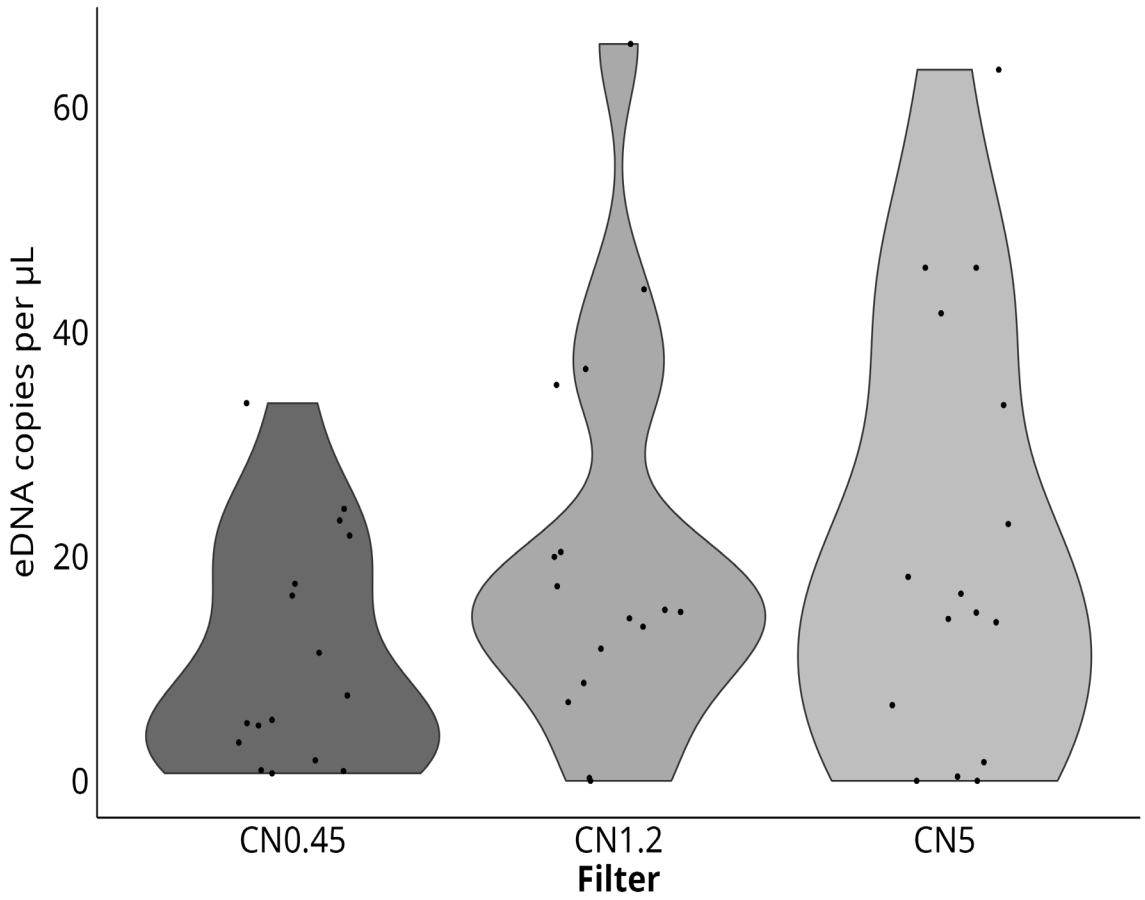


FIGURE 4. Number of eDNA copies per µL by filter type for water samples collected during Experiment 1, Big Otter Creek, Ontario, September 2021. For the violin plots, areas of greater width correspond to value ranges where data points are more concentrated, whereas narrower sections represent ranges where observations are sparser. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm.

Table 3.—Adjusted probability values resulting from the post-hoc Dunn's multiple comparison test for Experiment 1, Credit River, Ontario, to assess differences in DNA copies/µL among independent groups of biological samples collected from each location. The test followed a significant Kruskal-Wallis analysis, aiming to identify pairwise differences in median DNA copies/µL among filter types. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm. Adjusted probability values significant at the 5% level are denoted by an asterisk.

| Filter comparison | Credit River |
|---|---|
| CN0.45 - CN1.2 | $1.26 \times 10^{-8}$* |
| CN0.45 - CN5 | $2.26 \times 10^{-8}$* |
| CN1.2 - CN5 | $1.00 \times 10^{0}$ |
| CN0.45 - GF1.5 | $1.66 \times 10^{-3}$* |
| CN1.2 - GF1.5 | $4.37 \times 10^{-2}$* |
| CN5 - GF1.5 | $5.71 \times 10^{-2}$ |

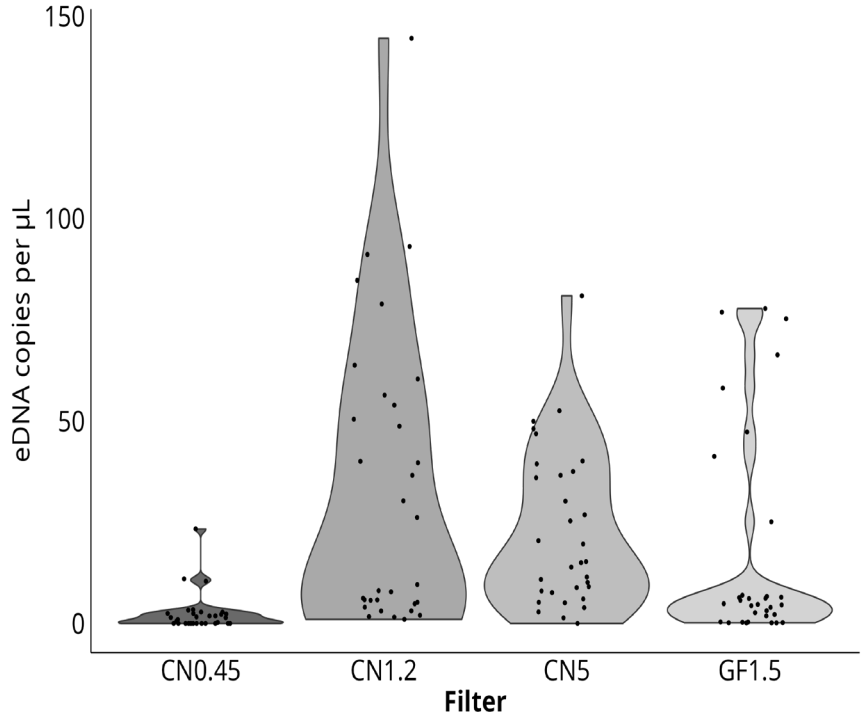


FIGURE 5. Number of eDNA copies/µL by filter type for water samples collected during Experiment 1, Credit River, Ontario, June 2022. For the violin plots, areas of greater width correspond to value ranges where data points are more concentrated, whereas narrower sections represent ranges where observations are sparser. For the filter-type abbreviations, the first two characters are the filter material, and the following number is the filter pore size in µm.

## Experiment 2: Comparing Two Filtration Devices For eDNA Sample Collection

The difference in frequency of inhibited samples between the two filtration devices was not statistically significant (GLMM, $P = 0.16134$) and was influenced by the river of collection. For the OSMOS aquatic eDNA sampler, 17.86% of the 84 non-negative-control samples were inhibited compared to 23.81% of the 84 non-negative-control samples collected with the peristaltic pump (Figure 6). The standard deviation of the GLMM was 15.06 with river as a fixed effect, which suggests that the log odds of inhibition vary greatly among river systems.

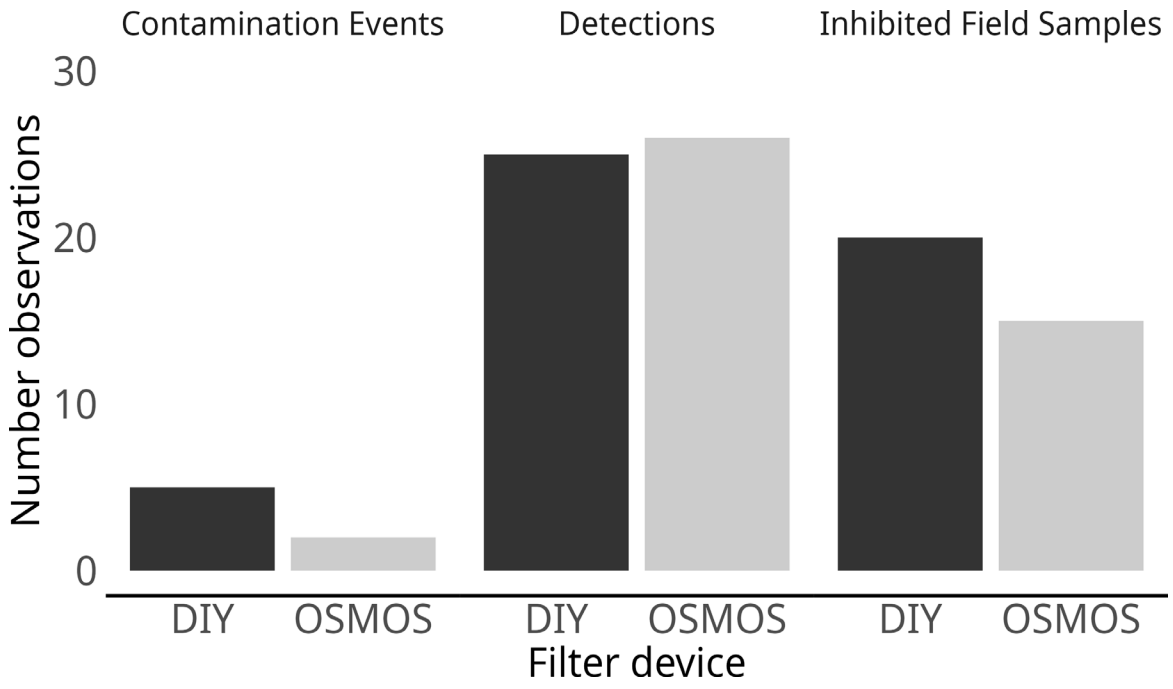


FIGURE 6. Number of eDNA contaminated field negative controls, detections, and inhibited samples for the OSMOS aquatic eDNA sampler and peristaltic do-it-yourself pump filtration devices that occurred at 28 stations across 9 tributaries in the U.S. and Canada during the fall of 2021.

For contamination, 2 of the 28 field negative controls collected with the OSMOS aquatic eDNA sampler were contaminated (7.14%), and five of the 28 field negative controls collected with the peristaltic pump were contaminated (17.86%) (Figure 6). However the GLMM analysis suggests the difference was not statistically significant ($P = 0.2099$).

There were no statistically significant differences in the frequency of positive non-negative-control samples between the OSMOS aquatic eDNA sampler and the peristaltic pump after samples from stations with contaminated field negative control and inhibited field were

excluded from the GLMM analysis ($P = 0.831$). The frequency of positive non-negative-control samples for the OSMOS aquatic eDNA sampler was 57.79% of 45 samples compared to 55.56% of 45 samples for the peristaltic pump (Figure 6).

The three-fold dilution series included with each qPCR run amplified with varying quality: some amplified as expected, while others showed variations in Cq value. While not ideal, the conclusions drawn from the analysis are based on qualitative presence-absence and do not depend on the data from the dilution series. Additionally, there is no evidence that the sensitivity of the analysis changed among runs, as the mean and variance for each run do not differ significantly. For the analysis of qPCR plate variance, see Supplementary Information S5: Analysis of Cq Mean and Variance by Run for the Filtration Device Comparison Experiment available in the online Supplement of this publication.

## Experiment 3: Determining the Attenuation of Adult Sea Lamprey eDNA Signal in a Great Lakes Tributary

Sea Lamprey eDNA detections were observed in field samples during the first (July 4) and second (July 18) sampling activities but not during any of the three sampling dates in August (Figure 7). Importantly, there was no contamination detected in any of the field negative controls, lab negative controls, or qPCR no-template controls. Furthermore, the consistent amplification of positive controls across all qPCR runs provides strong evidence that the absence of Sea Lamprey eDNA in the latter samples is likely due to the actual absence of eDNA rather than issues with the qPCR reagents or conditions. This pattern suggests that the spawning Sea Lamprey eDNA signal attenuated between the second and third sampling activity.

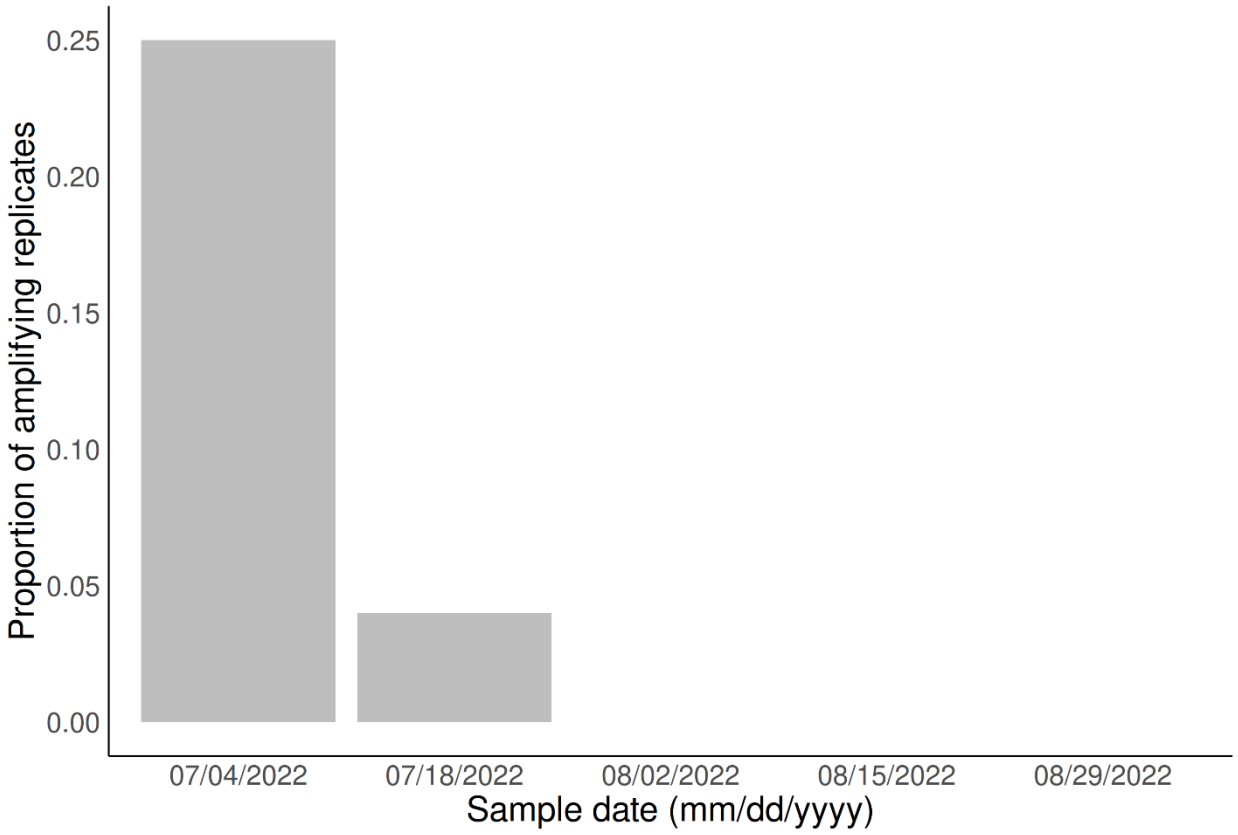


FIGURE 7. The proportion of qPCR replicates that registered a Cq value for eDNA samples of adult Sea Lamprey collected in Experiment 3 from the Humber River, Ontario, on five dates in 2022. Proportion of qPCR replicates amplifying is shown for each date the samples were collected; the last adult spawning Sea Lamprey was trapped on June 17, 2022.

# DISCUSSION

By synthesizing the results from the three experiments into a comprehensive eDNA sampling protocol, we hope to provide a practical solution for ecosystem managers seeking to survey larval Sea Lamprey with eDNA methods. Our thoroughly tested protocol aims to promote wider adoption of eDNA collection methods for management applications. Postponing sampling for a minimum of six weeks after the spawning run ends, using a 5.0-µm CN filter in combination with the OSMOS aquatic eDNA sampler (or the backup peristaltic pump), and following rigorous negative control and sterile-technique protocols represent substantial advancements in determining the most effective eDNA methods for monitoring larval Sea Lamprey.

The 5.0-µm CN filter was selected for routine use, despite the absence of a statistically significant difference in median DNA yield between the two consistently highest-performing filters (1.2-µm and 5.0-µm CN) from either sampling location. This decision was based on additional considerations, particularly the expected reduction in clogging risk due to its larger pore size. Along this line, using a larger pore-size filter may improve eDNA yield. Research by Thomas et al. (2018) suggests that high filtration pressures can lead to reduced eDNA retention on filters. Therefore, using a larger pore-size filter may decrease the likelihood of high filtration pressures, potentially enhancing eDNA retention. The 5.0-µm CN filter was also determined to be the best choice for the OSMOS aquatic eDNA sampler's pump performance (M. Hall, Halltech, personal correspondence), which may help lower filtration pressure, enhancing DNA retention and reducing stress on the filtration device.

Our study supports the findings of Toshiaki et al. (2020), which concluded that reduced eDNA yields were not caused by larger filter pore sizes, despite the concern that large pore sizes may allow smaller DNA fragments to pass through the filter (Turner et al. 2014). Research has indicated that factors beyond pore size significantly influence DNA yield. For example, the choice of extraction kit and its interaction with filter material may play a significant role. Our use of the DNeasy® Blood & Tissue extraction kit, as shown in the study by Hinlo et al. (2017), may result in higher DNA yields when combined with CN filters instead of GF filters. This finding may explain why the 1.2-µm CN filter performed notably better than the 1.5-µm GF filter, despite their similar pore sizes. In our study, CN filters with a pore size of 1.2 µm or greater consistently captured the highest amount of eDNA and were chosen for subsequent use in Sea Lamprey eDNA monitoring. However, it is important to note that this choice, despite evidence of reduced clogging risk and efficient pump performance, may not be applicable for all species. Contexts may exist where smaller pore sizes, ranging from 0.2 µm to 0.6 µm as suggested by Eichmiller et al. (2016), could be more appropriate, and the specifics of the target organism and sampling environment should be taken into consideration when selecting a filter.

No significant performance differences were observed between the OSMOS aquatic eDNA sampler and the do-it-yourself peristaltic pump, indicating that both devices can be reliably used for Sea Lamprey eDNA sampling. For subsequent experiments, the OSMOS sampler was chosen as the primary device for field sample collection, while the peristaltic pump was included as a backup in the equipment list. In the field, equipment can be susceptible to damage, leading to temporary malfunctions in both filtration devices. Consequently, a backup filtration device became essential. The peristaltic pump is the more budget-friendly option, priced at approximately Can$800 for the pump alone and Can$1,200–$1,800 for the complete package including the power drill, replacement battery, and tubing. The peristaltic pump is also lighter and more compact, which might be desirable for remote sampling locations. However, feedback from field crews indicated that they generally favored the OSMOS sampler, which costs approximately Can$8,500-10,000; they found it easier to use and better suited for routine monitoring. The OSMOS is also faster when it comes to the filtration time of individual samples, although this time savings was relatively minor in the course of the entire field sampling workflow. A multitude of eDNA sample filtration devices, including other autosamplers, are available on the market; users should select and test filtration devices based on project needs and budget constraints. Another crucial point to address is the handling of inhibition in eDNA samples. As shown in the results, samples

collected using both filtration devices were affected by inhibition. Commercial products, such as the OneStep PCR Inhibitor Removal Kit (Zymo Research), are often used to remove PCR inhibitors from eDNA samples, although their efficacy can be inconsistent (Loeza-Quintana et al. 2021). Noncommercial methods of PCR inhibitor removal have also been employed in eDNA research (Milián-García et al. 2021).

Trapping data from the Humber River indicated that the highest number of adult spawners entered the system between May 8 and May 30, 2022, and the last adult spawning Sea Lamprey was trapped on June 17 (R. Booth, unpublished data). Sea Lamprey eDNA could still be detected (at lower rates) by July 18, but the lack of detections by August 2 indicates that the spawner eDNA signal had left the system within 4–6 weeks after the last adult Sea Lamprey capture. This pattern aligns with findings of Gingera et al. (2016), although without the confounding effects from a larval signal. Most Sea Lamprey spawning occurs in June–July, but the timing varies considerably across the Great Lakes. Adults usually begin entering tributaries when the water temperature reaches 15°C, and spawning can occur as early as May or as late as September (Manion and Hanson 1980). Therefore, the absolute timing (i.e., calendar date) of eDNA sampling for larval assessment will differ depending on regional spawning time but should be at least six weeks after spawning ends. Beyond the differences in the timing and duration of Sea Lamprey spawning, various environmental conditions can influence eDNA degradation and the duration that spawner eDNA remains viable for analysis. For instance, eDNA degradation may be affected by factors such as water temperature, ultraviolet B (UV-B) levels, and pH (Strickler et al. 2015), and dispersion will be impacted by river flow and hydrology (Harrison et al. 2019). Additionally, environmental variables, particularly temperature, are likely to affect the rate of eDNA production by spawning Sea Lamprey (Lacoursière-Roussel et al. 2016; Stewart 2019). To improve our estimates, future research endeavors should investigate how biotic and abiotic environmental factors, including water temperature, UV-B levels, and pH interact with eDNA and impact spawning Sea Lamprey eDNA production and attenuation.

While it is anticipated that Sea Lamprey eDNA from adult spawners will typically dissipate from a tributary about 4–6 weeks after spawning concludes, it is important to consider the potential impacts of climate change on this time frame. Lennox et al. (2020) have projected that climate change could alter the life history of invasive Sea Lamprey and, as surface water temperatures in the Great Lakes continue to rise (Trumpickas et al. 2009), the timing and duration of Sea Lamprey spawning may change. In addition to these factors, climatic changes are also expected to modify the peak daily flow of Great Lakes tributaries (Byun et al. 2019). However, the precise effects of these changes on the attenuation of the eDNA signal after the spawning run remain uncertain. Understanding these complex interactions between environmental variables and eDNA dynamics is crucial for accurate monitoring and management of Sea Lamprey.

Contamination is a common and ongoing concern in eDNA studies (Sepulveda et al. 2020) and, as reflected in our results, this study was also impacted by contamination. Although we adhered to rigorous protocols for collecting negative controls and decontaminating equipment, it is important to further improve these methods to reduce the incidence of contamination in future research. Several guidelines for eDNA sample collection in aquatic systems have been developed (Carim et al. 2016; Welsh et al. 2019; Amberg and Hunter 2022; Vazquez et al. 2023), providing valuable resources for strategies to mitigate contamination. Based on the results of our study, contamination was observed only in the field blanks and was absent from the DNA extraction and qPCR negative controls. Therefore, our efforts should focus on maintaining sterile techniques during field collection. Potential ways to reduce contamination include increased use of sterile single-use materials, adopting a tiered approach to decontamination (Vazquez et al. 2023), and providing additional training and practice for field crews.

In summary, we recommend initiating eDNA sampling for larval Sea Lamprey at least six weeks after the estimated regional spawning period, using a 5.0-µm CN filter in combination with the OSMOS aquatic eDNA sampler (or the peristaltic pump as a backup), and adhering rigorously to negative control and sterile-technique protocols. Other eDNA samplers are available and could be used for eDNA collection; however, significant differences can exist, and their performance would need to be tested (e.g., Nolan et al. 2023) before using. The protocol for eDNA sample collection is presented in the Appendix. Our proposed approach considered not only the ecology of the target species, but also the need for rigorous contamination prevention and the nuances of collection and filtration methods, thus representing a significant advancement in optimizing eDNA methods for larval Sea Lamprey monitoring. This well-validated approach improves the efficacy and reliability of eDNA-based monitoring efforts, providing a valuable tool for the sustainable management of aquatic ecosystems and addressing critical gaps in the field, thereby supporting long-term sustainability.

## ACKNOWLEDGMENTS

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# APPENDIX

## Standard Operating Procedure: Environmental DNA Field Sample Collection for Invasive Sea Lamprey Monitoring

## Document Control

| Version | Date | Description of changes | Authors |
|---|---|---|---|
| 1.0 | January 1, 2024 | Initial creation | Cameron D. Brown, Robert H. Hanner, Margaret F. Docker |

### 1. Purpose and Scope

The primary goal of this Standard Operating Procedure (SOP) is to standardize the collection of environmental DNA (eDNA) samples for monitoring the invasive Sea Lamprey *Petromyzon marinus* in Great Lakes tributaries. This SOP provides a detailed, step-by-step methodology for eDNA sample collection, ensuring accurate and reliable eDNA data collection that is essential for decision making.

This SOP specifically addresses the collection of eDNA samples targeting the larval stage of the Sea Lamprey. It is applicable to the environmental conditions present in Great Lakes tributaries. For adaptations of this protocol to different environmental conditions, life stages, or target species, consultation with an expert is advised, as variations to the sampling parameters (e.g., life stage, sampling environment, target species) may necessitate modifications to the procedure.

### 2. Responsibilities

While many individuals may participate in the eDNA sample collection process, a minimum of two people is required for samples to be collected effectively. The roles are data recorder and sample handler.

**Data Recorder**
The data recorder is responsible for accurately capturing all necessary data during eDNA field sample collection. This includes recording essential details on the field data sheet, such as site name, station name, date, sample ID, time of collection, and volume of water filtered. The data recorder must also ensure that photographs of the physical sample and sampling station, if required, are taken and appropriately documented. Although others may assist, one individual must be assigned this responsibility to ensure the accuracy and completeness of all recorded data.

**Sample Handler**
The primary responsibility of the sample handler is to manage the filter used for collecting

eDNA from the water. This includes placing the unused filter into the filtration device before collection and transferring the filter into the appropriately labeled coin envelope preserved in desiccated silica after sample collection. Proper handling is crucial to avoid contamination or damage to the filter, which could significantly reduce the likelihood of obtaining usable results.

**Additional Duties**

Additional tasks in eDNA sample collection include setting up and dismantling equipment, collecting supplementary data (e.g., water-quality measurements), and managing plastic waste. After the primary responsibilities of data recorder and sample handler are assigned, field teams are encouraged to distribute additional duties among themselves as they see fit. This distribution should occur in a manner that ensures efficiency and adherence to the SOP.

## 3. Materials and Equipment Sampling Checklist

Common items for both OSMOS and DIY Sampling (GEN-FISH 2022b, 2022d):

- Sterilized buckets: individually wrapped in plastic bags
- Tap water: stored in a sterilized container for the field negative control
- Backpack or field bin: for transporting supplies to the field location
- Sterile nitrile gloves: ensure sizes fit all team members
- Kimwipes™ or paper towel: for on-site sterilization or equipment maintenance
- ELIMINase™: for cleaning and decontaminating equipment
- First aid kit: fully equipped for emergency scenarios
- Garbage bags: for waste disposal
- Clipboard: to hold and manage field data sheets
- Field data sheets: for recording sampling data
- GPS: for accurate location tracking.
- Water chemistry probe (optional): for collecting water chemistry metadata
- Forceps and filters: sufficient for planned sampling, pack five extra as a buffer
- Coin envelopes: sufficient for planned sampling, plus additional spare envelopes
- Plastic bags: small and large, resealable, for sterile material storage and sample preservation
- Silica beads: 30 g per negative-control bag, 90-100 g per bag for biological replicates
- Labels for each coin envelope and station bag, plus blank extras
- Filters, forceps, coin envelopes, and silica beads packed according to the infographic below (Figure A.1).

For a visual summary of materials needed across sampling stations, refer to Figure A.2.


FIGURE A.1. Infographic showing the consumable materials required for OSMOS sampling: the set includes sterile forceps with a preloaded filter canister, three filters for biological replicates, a sterile bucket sealed in a garbage bag for collection of the negative control, three coin envelopes filled with silica for the biological replicates, and one coin envelope with silica for the negative control. It is essential to fill coin envelope bags with the specified amounts of silica beads—30 g for the negative-control bag and 90-100 g for each biological-replicate bag—to ensure proper preservation of eDNA samples (GEN-FISH 2022d).

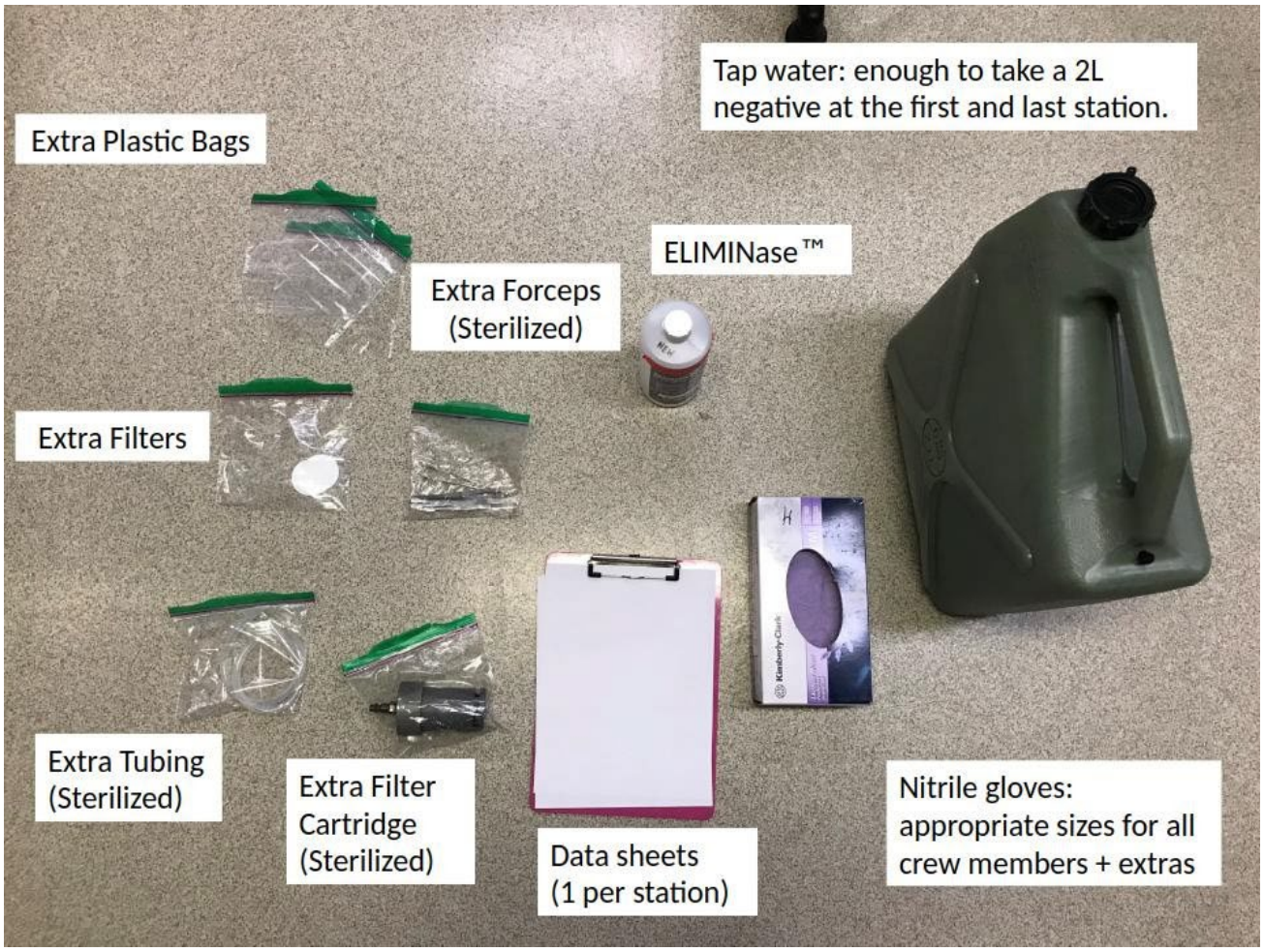


FIGURE A.2. Infographic showing the materials needed cross-station: sterilized container carrying enough tap water to collect a 2L negative at the first and last sampling station, nitrile gloves in appropriate sizes for all team members plus extras, five UV-irradiated plastic bags containing extra filters, five extra sterilized forceps, one extra filter cartridge (sterilized) for the OSMOS, extra tubing for peristaltic pump (sterilized), additional resealable plastic bags (both large and small sizes). Also bring field data sheets (1 per station) and ELIMINase™ (GEN-FISH 2022b, 2022d).

Specific items for OSMOS sampling (GEN-FISH 2022d):

- OSMOS
- Telescoping pole for OSMOS
- Inflow tube for OSMOS
- Outflow tube for OSMOS
- OSMOS tripod
- OSMOS batteries: ensure they are fully charged
- Filter canisters: one for each planned sampling station
- One additional filter canister: as a backup in case of loss or damage

See Figure A.1 for a visual guide of the consumable materials needed for aquatic eDNA sampling with the OSMOS Aquatic eDNA Sampler.

Specific items for DIY peristaltic pump sampling (GEN-FISH 2022b):

- Reaching pole: or suitable alternative, such as a dip net, for sample collection
- Flat-head screwdriver
- Wrench
- Filter holder: part of the peristaltic pump kit
- Cordless drill
- Nalgene® bottles: ensure an adequate number are available for planned sampling
- Drill battery: charge fully before leaving for sampling site
- Duct tape: for securing components or making quick repairs in the field

See Figure A.3 for a visual guide of the consumable materials needed for aquatic eDNA sample collection with the DIY peristaltic pump.


FIGURE A.3. Infographic showing the consumable materials required for the DIY peristaltic pump sampling. The set includes one coin envelope in desiccated silica for the negative control, and three coin envelopes in desiccated silica for the biological replicates. Additionally, there are four sterile forceps, four filters for collection of the biological replicates and the negative control (NC), sterile tubing, and a Nalgene® bottle. For adequate preservation, the sealable plastic bag containing the coin envelopes should be filled with silica beads—30 g for the negative-control bag and 90-100 g for the bag containing the biological replicates (GEN-FISH 2022b).

**Important Note**
Collecting eDNA samples often incurs significant costs and time commitments, primarily due to the transportation of personnel to and from sampling stations. To ensure efficient sample collection and to mitigate the risk of equipment failure, it is advised that sampling teams prepare and bring both types of filtration devices on the sampling venture. This strategy helps ensure successful sampling, even if one of the devices encounters mechanical issues.

### 3.1. Material Preparation

Prior to eDNA sampling using the OSMOS sampler, a rigorous sterilization process is required to ensure the integrity of collected samples. Prior to any sampling event, perform the following steps:

**Cleaning of OSMOS Canisters and Forceps**
OSMOS filter canisters and forceps are to be cleaned using a standardized protocol. A bucket capable of holding a minimum of 3 L of water (for the field negative control) is sterilized by either soaking it in a 10% bleach solution for 30 min or wiping it down with ELIMINase™. The sterilized bucket is then sealed inside a clean garbage bag.

**Sterilized Jerrycan**
A jerrycan (or similar water container) is sterilized by rinsing the inside with 10% bleach solution and then filling the container with tap water to transport the water used for the field negative control to the sampling station.

**Preparation of Consumable Materials**

Forceps, filters, and gloves are placed into separate resealable plastic bags. The plastic bags containing the forceps and gloves are then subjected to 15 min of ultraviolet (UV) irradiation (GEN-FISH 2022b, 2022d). Filters should not be exposed to UV irradiation as this can damage the filters. See Figure A.4 for the recommended organization of consumables for eDNA sample collection with both the OSMOS Aquatic eDNA Sampler, and the DIY peristaltic pump.


FIGURE A.4. Infographic showing the recommended organization of consumables for eDNA field sample collection with the OSMOS and the DIY peristaltic pump. Each station's consumables are placed into a single large plastic bag, clearly labeled with the station ID for easy identification and organization: A = OSMOS sampling bag containing a loaded filter cartridge, forceps, three filters for the biological replicates, and three coin envelopes in desiccated silica for the biological replicates; B = DIY peristaltic pump sampling bag containing sterile forceps, tubing, four filters, three coin envelopes for desiccated silica for the biological replicates, and one coin envelope in desiccated silica for the negative control (GEN-FISH 2022b, 2022d). Such organization is critical for efficient and accurate eDNA sample collection.

**Silica Bead Storage**
Resealable plastic bags are filled with desiccated silica beads, with each bag containing 30 g of silica per coin envelope (GEN-FISH 2022b, 2022d). One coin envelope per plastic bag is used for field-negative controls, while three envelopes per plastic bag are used for each of the biological replicates.

**Labeling**
Coin envelopes are labeled with a unique sample code and sample bags are labeled with unique station codes.

**UV treatment**
All materials, including silica beads, coin envelopes, and labels, are subjected to 15 min of UV irradiation within a sterilized UV cabinet before use in the field (GEN-FISH 2022b, 2022d).

**Decontamination Protocol for OSMOS**

OSMOS unit:
- Set volume and filter 10 L of tap water through the unit.
- Wipe down OSMOS unit, telescoping pole, tripod, and inflow tubing with ELIMINase™ and paper towel or bleach wipes. Rinse with tap water or wipe down with wet paper towel. Look for scrapes on the OSMOS unit, fraying and ripping of backstraps, and permanent marks on the OSMOS control panel glass.
- Soak outflow tubing in 1% bleach for 30 min.
- Thoroughly rinse the tubing and dry with a paper towel, then hang to air-dry.

Filter cartridge:
- Separate the filter housing into its three components.
- Soak rubber gaskets in ELIMINase™ for 5 min.
- Submerge inlet- and final-stage components in 10% bleach for 30 min.
- Rinse each component three times in distilled water; allow draining between each rinse.
- Allow to air-dry.
- Repeat rinsing if bleach residue is present.
- Rinse rubber gaskets with distilled water.

Forceps:
- Soak in 10% bleach for 30 min, rinse three times with distilled or tap water, then air-dry. This can be done with cartridges.

# 4. Procedure

### 4.1 Station Workflow for eDNA Sample Collection

The following workflow outlines the sequential steps for eDNA sample collection at each station:

1. Set up the OSMOS or DIY peristaltic pump system at the station.
2. Filter the negative-control sample (this step is performed only at the first and last station).
3. Filter biological-replicate samples.
4. Take a photograph of the OSMOS/DIY setup to document the setup conditions.
5. Collect water-quality data or other additional environmental metadata.
6. Take photographs of the site to visually document the sampling environment.
7. Enter all necessary information into the field data sheet.

Note: Refer to the Halltech OSMOS manual and GEN-FISH guidance documents (GEN-FISH 2022b, 2022d) for how to properly assemble and disassemble each filtration device.

**Workflow for Collection of Individual Sample**

Maintaining sterility during and after sampling is crucial to prevent contamination. Follow these guidelines:

- Handle the filter cartridge/pump head, filter, coin envelope, and forceps only with sterile gloves.
- Be aware of contamination sources, including the outside of plastic bags, the OSMOS unit, telescoping pole (including brass fitting), drill, reaching pole, clothing, and the ground.
- If your gloves, forceps, or filter come into contact with any contamination source, they must be considered not sterile and replaced immediately.

**Sterile Sample Handling**

The filter should always remain at least two sterile layers away from any potential source of contamination, such as filter, forceps, gloves, hand (source of contamination), or filter, coin envelope, plastic bag, larger sample bag for transport (source of contamination).

**Material Separation**

Materials that come into direct contact with the filter (forceps, gloves, coin envelopes, filtration canisters) are never shared across sampling stations and are always kept separate from materials used at other stations.

### 4.3 Sample Collection Procedure

#### 4.3.1. Overview of Individual Sample Collection Procedure

The individual eDNA sample collection procedure can be summarized in six key steps:

1. **Preparation**: Review the sample collection steps and set up the DIY peristaltic pump or OSMOS according to the manual upon arrival at the sampling station.

2. **Preparing the Negative Control**: Collect 1–2 liters of tap water in a clean Nalgene® bottle or sterilized bucket to serve as a negative control sample.

3. **Filter Installation**: Install a new filter into the pump head or filter housing using sterilized forceps while wearing gloves to maintain sterility.

4. **Sample Collection**: Use the OSMOS or DIY peristaltic pump to filter the negative control sample through the installed filter.

5. **Storing the Filter**: After filtration, carefully remove the filter, photograph it for documentation, fold it appropriately, and place it into the designated coin envelope to avoid contamination.

6. **Collecting and Filtering Biological Samples**: Repeat the filtering process for the biological water samples, following the same steps as above, ensuring sterility and proper handling for each sample.

This overview provides a quick reference of the sample collection process. Below is a detailed, step-by-step procedure.

#### 4.3.2. DIY Peristaltic Pump Procedure (GEN-FISH 2022b)

**Preparation**

- Start by reviewing the basic steps for sample collection using the DIY peristaltic pump.
- Set up everything according to the manual upon arrival at the sampling station.
- Ensure the DIY peristaltic pump is correctly and appropriately assembled.

**Preparing the Negative Control**

- Remove the clean Nalgene® bottle from the plastic transport bag.
- Open the Nalgene® bottle carefully while wearing gloves.
- Have a second person use a sterilized container to fill the Nalgene® bottle with tap water.
- Fill the bottle up to 1 L.

**Filter Installation**

- Unscrew the pump head (filter handler wearing gloves).
- Hold the pump head during the process with the second person's assistance.
- Change gloves for sterility filter handler).
- Carefully take a clean filter from the filter bag using fresh gloves.
- Grab a filter from the filter bag using sterilized forceps.
- Carefully place the filter into the pump head using the forceps.
- Place the orange O-ring over the top of the filter and the pump head.

Securely screw the pump head shut. See Figure A.5 for an important note about differentiating the filter from the paper filter divider.

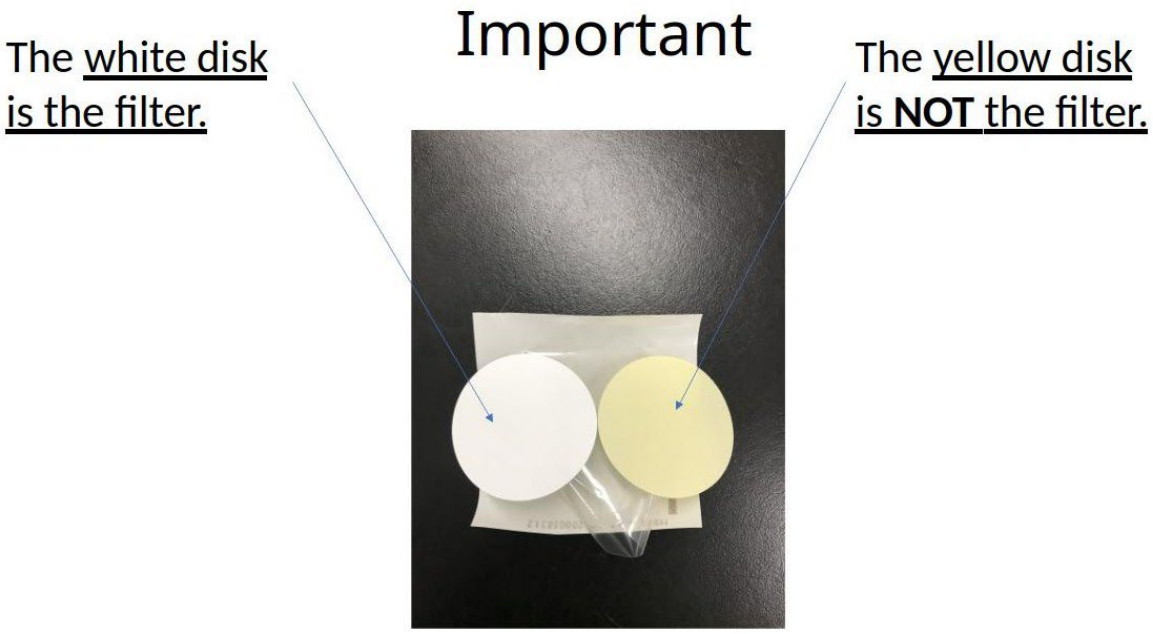


FIGURE A.5. The white disk is the filter. The yellow disk is NOT the filter. The color of the filter and the paper filter divider may differ depending on the brand and type of filter used. Always double check the product details.

**Sample Collection**

- Be conscious of the surrounding space during the process. To avoid contamination, ensure the pump head does not swing into and hit any objects like waders or the ground to avoid contamination.
- Have one person wearing gloves hold the Nalgene® bottle filled with tap water.
- Have a second person hold and operate the filtration system with the drill.
- Coordinate roles and actions to manage the equipment effectively during sample collection.
- Run the drill at a slow, steady pace to avoid tearing the filter.
- Drain and filter the entire 1 L of tap water from the Nalgene® bottle.
- Stop the process after the first liter is filtered.
- Refill the Nalgene® bottle with tap water from the sterilized water container.
- Filter the entire second liter of tap water, ensuring a total of 2 L has been pumped through the filter.

**Storing the Filter**

- Have the non-filter handler hold the filtration apparatus after sample filtration.
- Have the filter handler carefully unscrew the filter head.
- Remove the orange O-ring from the pump head using sterilized forceps.
- Have the secondary person take a photo of the filter in its current position for documentation.
- Have the filter handler then open the appropriate negative-control sample bag and open the coin envelope.
- Prevent contamination by only touching the coin envelope with the forceps.
- Place the filter inside the coin envelope to ensure minimal contact and contamination risk.

**Collecting and Filtering Biological Samples**

- Reuse the Nalgene® bottle for biological samples if it is clean, following the same procedures as for the negative control.
- Secure the Nalgene® bottle to the reaching pole or other suitable devices as per the instructions.
- Position the opening of the Nalgene® bottle facing upstream while collecting samples.
- Submerge the bottle about 15 cm below the waterline, stretching out as far as possible into the water.
- Focus on collecting samples from flowing water rather than still water or eddies.
- Filter each biological sample using the same steps as in the Sample Collection Procedure.
- Follow the Storing the Filter procedure after filtering each biological sample. However, instead of placing the filter in the negative-control bag, use the designated coin envelopes and sample bag for each biological sample.
- Repeat the process for each biological sample, ensuring fresh gloves and sterilized forceps are used each time.
- Continue the process until all required biological samples have been collected and filtered.

**Additional Notes**

- When securing parts in place during the process, ensure they are firm but avoid overtightening to prevent breakage.
- The non-filter handler is responsible for managing the Nalgene® bottle used for the negative control, which can be reused for subsequent biological samples at that sampling station.
- Remember to record the estimated amount of water filtered and the time the filter photo was taken for each sample.

### 4.3.3 OSMOS Procedure (GEN-FISH 2022d)

**Preparation for OSMOS Sampling**

- Begin by setting up the OSMOS by the riverside, following the setup protocols provided in the Halltech OSMOS manual.
- Ensure the data recorder has noted station ID, latitude, longitude, additional metadata, and time of arrival.
- Perform any required pre-priming as dictated by the OSMOS model being used (GEN-FISH 2022c).

**Preparing the Negative Control**

- Take the container (such as a plastic bucket) out of its clean storage bag.
- Use a jerrycan or other sterilized water container to fill the bucket with about 3 L of tap water.

**Setting Up the OSMOS**

- Take a sterilized filter housing from its sealed plastic bag.
- Firmly connect the filter housing to the OSMOS and its telescoping pole.
- Always handle the OSMOS canister with clean plastic gloves to minimize the risk of contamination.

**Installing the Filter**

- Open the OSMOS canister.
- Remove the bottom part of the canister to access the filter placement component.
- Remove the O-ring from the inside of the canister using sterile forceps.
- Carefully place the filter flat on the metal mesh inside the canister.
- Replace the O-ring on top of the filter and reassemble the canister.
- Ensure all components are secure, including the connection of the canister to the telescoping pole.
-

See Figure A.5 for an important note about differentiating the filter from the paper filter divider.

**Filtering the Negative Control**

- Lower the canister into the bucket containing tap water using the telescoping pole.
- Be careful to avoid having any part of the telescoping pole, including the brass knuckle, touch the waterline as this poses a contamination risk; See Figure A.6 for proper positioning of the filter cartridge to avoid contamination during negative control sampling.
- Follow the instructions in the Halltech OSMOS manual to filter two L of water from the negative-control bucket.

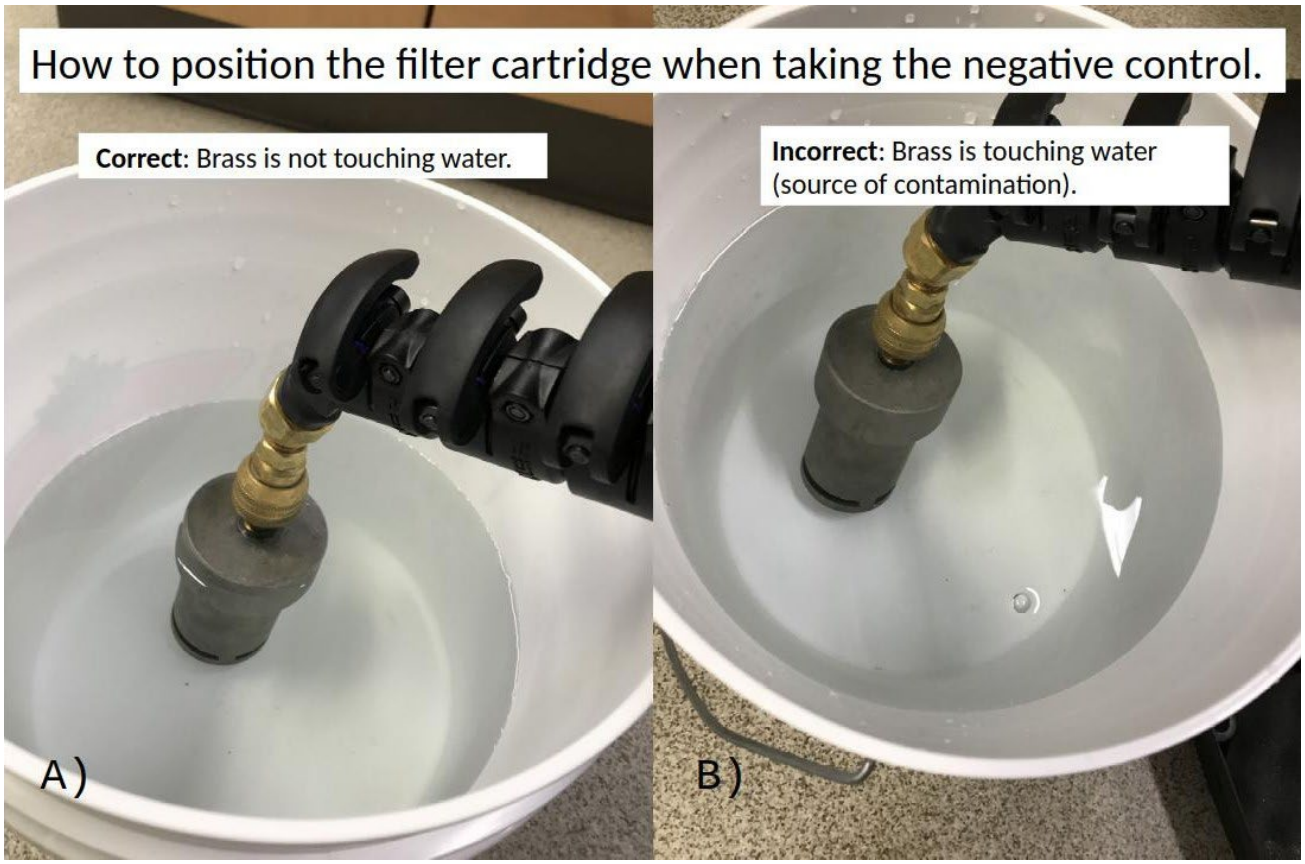


FIGURE A.6. How to position the filter cartridge correctly when taking the negative control: A = correct position—the brass on the telescoping pole is not the touching water. B = incorrect position—the brass on the telescoping pole (source of contamination) is touching the water.

**Storing the Filter**

- Invert the OSMOS telescoping pole to drain the water after filtration.
- Carefully remove the bottom portion of the OSMOS canister to access the filter.
- Using sterile forceps, remove the O-ring so the filter is fully visible.
- Have a secondary person take a photo of the filter for documentation.
- Fold the filter in half with the dirty side facing in and place it in the appropriately labeled coin envelope.
- Seal the coin envelope and the plastic bag.

**Collecting and Filtering Biological Samples**

- Repeat the Filter Installation steps for each biological sample.
- Extend the telescoping pole as far into the river as possible to collect the sample from flowing water.
- Lower the OSMOS canister so that the bottom of the canister is about 15 cm below the waterline.
- Notice that, for biological samples, it is acceptable for the telescoping pole to enter the water.
- Use the default OSMOS settings to filter 2 L of water from the river.
- Follow the same Storing the Filter procedure as for the negative control.
- Record sample summary information including average sampling time, flow rate, total volume collected, and filter photo time.
- Repeat this process for each biological sample, ensuring sterilized gloves and forceps are used for each sample.

### 4.3.4 Key Parameters for Sampling

For consistent and accurate biological-replicate sampling, adhere to the following key parameters:

- Sample depth: The sample depth for biological replicates should be approximately 15 cm. This depth corresponds to the waterline being just above the first brass knuckle on the telescoping pole.
- Sample volume: The sample volume should be 2 L. Always use the default settings on the OSMOS system for this purpose.
- Pole extension: Extend the telescoping/reaching pole to its maximum length or to the center of the river, whichever is closer to your current position.
- Sampling location: Avoid sampling in eddies or still water. Always aim to sample from the flowing part of the river.

Collecting eDNA samples in accordance with these parameters is crucial for consistent and accurate data collection.

## After-Sampling Procedures

### Sample Storage and Disposal

1. Place all sample storage bags (which include used filters, coin envelopes, and silica) into a large resealable plastic bag. Make sure this bag is labeled with the site ID.
2. Used filter cartridges, forceps, gloves, and other contaminated materials should be disposed of in the garbage bag that was originally used to store the bucket.

**Field Data Sheets**
Field data sheets should be completed for each sampling-station visit. Record site information, crew details, date, weather conditions, water-quality parameters, volume filtered, and time of filter photographs.

**Photographic Documentation**
It is recommended that a series of photos be taken during each sampling event, capturing various angles of the sampling station, including the OSMOS aquatic eDNA sampler or DIY peristaltic pump during sampling. For guidance on photographing the filter, refer to Figure A.7. Ensure the rubber gasket is removed to fully display the filter, as shown in the left panel.


FIGURE A.7 A = this is the correct image; the rubber gasket has been removed, and the filter is completely visible. B = this is the incorrect image; the rubber gasket is still on the filter.

**Sample Storage**
After returning from the field, the filters are kept within the coin envelopes, and they (along with the desiccated silica in the labeled sample bag) should be stored at -20˚C.

### 4.3. Troubleshooting and Contingencies

**When to Retake a Sample?**

There are specific instances during the sampling process when a sample must be retaken to ensure the integrity of the results:

1. The filter gets dropped or is otherwise contaminated.
2. The filter cartridge or pump head gets dropped during filtration.
3. A large tear in the filter occurs during sampling (it's ok if the filter tears while folding it).

In the event that any one or more of these three circumstances occur, do not attempt to save the filter. Instead, discard it and start again with a clean spare.

**Troubleshooting Filter Clogs**

During the sampling process, it is possible to encounter filter clogging. This section outlines the steps to identify and document this issue for both the OSMOS and DIY peristaltic pump.

- OSMOS: The filter is considered clogged when the average flow rate reduces to less than 0.05 L/min for a duration of 1 min.
- DIY: The filter is considered clogged if the pump is running but water has stopped exiting the pump head.

After a filter is determined to be clogged, proceed with the following steps:

1. Place the clogged filter in its designated coin envelope.
2. Make a note on the data sheet indicating that the filter has clogged (include the filter code on the coin envelope in the note).

**Troubleshooting Common Osmos Error Codes**

When operating the OSMOS, certain errors may be encountered. Below are the recommended actions for the most common errors:

- Error #1 (Pressure exceeded warning): Press the "Ent" button and continue to collect the sample until completion.
- Error #2 (No filtration): (1) Be patient—the OSMOS may start filtering after a couple of minutes; (2) if filtration does not commence, stop the run, adjust the pressure to 80-90 kPa, and attempt to filter again.

## 4. Training and Competency

While reference manuals and SOPs, such as this document, can be helpful in facilitating the consistent and accurate collection of environmental DNA samples, it is critical to remember that the environmental DNA sample collection is a highly technical physical process that requires many hours of in-person training and repeated practice—things that an SOP alone cannot substitute for.

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# Optimization of Methods for the Collection of Larval Sea Lamprey Environmental DNA (eDNA) from Great Lakes Tributaries: Supplementary Information

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# S1: Environmental DNA Sampling Field Data Sheet

| | | | |
|---|---|---|---|
| Project Name | Field-ready environmental DNA (eDNA) protocols and tools for Sea Lamprey assessment (2022) - GLFC | | |
| **Site Code** | | | |
| **Crew Members:** | | | |
| **Date (ddmmyy)** | | | |
| Arrival Time | _____:_____ (24h) | Departure Time | _____:_____ (24h) |

Sample Location

| | | | | |
|---|---|---|---|---|
| Waterbody Name | | | | |
| Latitude (dd.ddddd°) | | Longitude (-d.ddddd°) | | GPS Accuracy (m): |
| eDNA collection paired with conventional capture-based sampling? **(Yes /No)** | | | | Paired Site Code: |

**eDNA Sampling Details**

| Sample No. | Method (OS=OSMOS; D = DIY) | Sample Code<br>Matches code on coin envelope. Example: GLFC_OGON-001-S-L1, etc. | Volume (L) | **Average Flow Rate (L/Min)** | Time (sec) | Time photo of filter was taken | **Photo File Nos.** |
|---|---|---|---|---|---|---|---|
| Rep. 1 | | | | | | | |
| Rep. 2 | | | | | | | |
| Rep. 3 | | | | | | | |
| Rep. 4 | | | | | | | |

**Notes (*Did anything abnormal happen?*):**

| | |
|---|---|
| **R.1** | |
| **R.2** | |
| **R.3** | |
| **R.4** | |

| | |
|---|---|
| General | |

**Negative Control**

**Was a negative control collected? (Yes/No)**

| Sample No. | Method (OS=OSMOS; D = DIY) | Sample Code<br>Matches code on coin envelope.<br>Example: GLFC_HR-001-1-, etc. | Volume (L) | Average Flow Rate (L/Min) | Time (sec) | Time photo of filter was taken | Photo File Nos. |
|---|---|---|---|---|---|---|---|
| NC | | | | | | | |

**Stream Site Details**

| | |
|---|---|
| Distance from bank sampled (*Middle of river or max length of pole?*) | |
| Species observed at site? | |
| Above or below barrier? | |
| Larval habitat type at sampling site? (Type 1, 2 or 3?) | |

Site Characteristics

| Water Quality | Reading #1 | Reading #2 | Instrument |
|---|---|---|---|
| Air Temp (°C) | | | |
| Water Temp (°C) | | | |
| Electrical Conductivity (mS/cm) | | | |
| pH | | | |
| TDS (ppm) | | | |

**Weather Conditions**

| | |
|---|---|
| Briefly describe the weather conditions at the sampling site. (*Cloud cover, precipitation, wind*) | |

**Photographs** *If using phone please take horizontal photos.*

| Category | Photo File Nos. |
|---|---|
| Upstream | |
| Downstream | |
| Facing Sampling Site | |
| Behind Sampling Site | |
| Photo of OSMOS during sample (Ensure all components are in frame) | |

| | |
|---|---|
| Completed field sheets | |

Field Sheet Completed: Yes o No o

# S2: DNA Extraction Protocol

**Alterations from Qiagen Protocol:** Purification of Total DNA from Animal Tissues (Spin-Column Protocol)

Extraction Protocol One:

1. Volume of Buffer ATL increased from 180 µL to 450 µL.
2. Volume of Proteinase K increased from 20 µL to 450 µL.
3. Lysis occurs for 18-24 hours.
4. Use of Qia Shredder post lysis.
5. Buffer AL and 96% - 100% Ethanol volumes increased 200 µL to 400 µL.
6. Buffer AE volume reduced from 200 µL to 100 µL.

Extraction Protocol Two:
In addition to the modifications listed above…

1. Add 250 mg of glass beads before addition of sample and Buffer ATL
2. Disrupt filter before proteinase K digestion by placing in TissueLyser II and shaking for 2 minutes at a frequency of 30 (1/s).
3. Final elution volume of buffer AE was adjusted to 110 µL.

**Extraction Protocol One: Processing eDNA water filters using Qiagen DNeasy (Docker Lab UManitoba).**

*Qiagen DNeasy Extraction kit*
*NOTE: Extraction process takes two days for isolation and all liquid waste must be disposed of in DNA buffer waste collected*

***DAY 1 - Preparations***

***BLEACH***

1. Forceps

***UV***

1. Forceps
2. Petri Dishes
3. 1.5 mL tubes
4. Tube Racks
5. 1000 µL Pipette Tips
6. 100 µL Pipette Tips
7. XL Gloves
8. L Gloves
9. Ziploc Bag

***DAY 1 – Approximately 2 hours***

1. Wipe down all equipment, including centrifuge, vortex, outside of pipettes, commonly contacted surfaces with 80% Ethanol then ELIMINase.
2. UV treat the hood that contains all the necessary equipment such as gloves, petri dishes, forceps and Kim wipes.
3. Turn on the thermomixer and set temperature to 56°C, mixing or shaking speed to 350-400 rotations per minute (rpm).
4. Take out bags with eDNA filters from freezer and keep away from UV hood.
5. Place a (clean) filter into a plastic bag to serve as the extraction negative control.
6. Once UV station treatment complete, take out 1.5 mL tubes (# of tubes = # of eDNA filters plus one extra to serve as the negative control).
7. Add **450 µL** of **Buffer ATL** to each tube *(check to see if Buffer has salt solids in bottom by opening carefully in UV station – it salt deposits are present, place in water bath for 5 minutes before pipetting).*

8. Add **40 µL** of **proteinase K** to each tube.
9. Label 1.5 mL tubes with sample ID and date.
10. Start with the negative control sample. Leave the filter whole, unfold the filter on the petri dish and re fold it with the eDNA side facing outwards, then place into tube.
11. Wipe down counter with ELIMINase and Ethanol between sites.
12. Vortex thoroughly – for 15 to 20 seconds.
13. Place the 1.5 mL tubes in the thermomixer at 56°C, mixing 350-400 rpm.
14. Leave overnight or anywhere between 18-24 hours.

*At this point, if you want to process another batch, you can clean up all the equipment, wipe down pipettes with a Kimwipe with ELIMINase, and UV the PCR station.*

***DAY 2 - Preparations***

***BLEACH***

1. Forceps

***UV***

1. Forceps
2. 1000 uL Pipette Tips
3. 100 uL Pipette Tips
4. 1.5 mL Tubes
5. Qiashredders
6. Tube Racks

***PREPARE***

1. Collection Tubes x 2
2. DNeasy Tubes
3. LoBind Tubes

***DAY 2 – Approximately 3 hours***

***Set up station with DNA columns, Qia shredder columns, 1.5 mL tubes, collection tubes, forceps, ELIMINase and gloves***

1. Spin tubes down - 13,000 rpm.

2. Move each sample to a Qia shredder spin column by moving the filter with the sterilized forceps and pipette the rest of the liquid into the column.
3. Spin for two minutes at 11,000 rpm.
4. Remove columns and place liquid into a new 1.5 mL tube
5. Add **400 µL** of **Buffer AL** and **400 µL 100% ethanol** to each tube
6. Vortex tubes for 15-20 seconds.
7. Spin tubes down at 13,000 rpm.
8. Label the appropriate number of DNeasy Mini Spin Column or DNA collection columns (# of DNA columns = # of eDNA filters).
9. Pipette 600 to 700 µL of the mixture that is in the 1.5 mL tube along with the precipitate into the DNeasy Mini Spin Column.
10. Centrifuge at 8,000 rpm for 1 minute.
11. Discard the flow through into the waste container and place the tube back into collection tube.
12. Pipette the remaining mixture into the spin column and repeat centrifugation and discard flow through.
13. Keep the mini spin column and place into a *new* 2 mL collection tube and discard the used collection tube.
14. Heat Buffer AE on heating block at 70°C and set time for minimum 30 minutes without shaking.
15. Add **500 µL** of **Buffer AW1.**
16. Centrifuge for 8,000 rpm for 1 minute.
17. Discard the flow through into waste and discard the collection tube.
18. Transfer the mini column to a *new* 2 mL collection tube.
19. Add **500 µL** of **Buffer AW2.**
20. Centrifuge at 14,000 rpm for 3 minutes.
21. Discard the flow through and collection tube.
22. Keep the DNeasy Mini Spin Column.
23. Preheat Buffer AE for minimum 10-15 minutes at 70°C.
24. Label the appropriate number of 1.5 mL Lo-Bind tubes.
25. Transfer the DNeasy Mini Spin Column into each 1.5 mL LoBind tube.

26. Pipette **100 µL** of **HOT Buffer AE** into the spin column. *Make sure you're pipetting directly onto the filter membrane.*
27. Let tubes sit at room temperature for 5 minutes.
28. Centrifuge at 8,000 rpm for 1 minute.
29. **DO NOT DISCARD THE FLOW THROUGH - IT'S THE DNA.**
30. Discard the mini spin column and close the tube.

If you're going to be running an assay with DNA in the next few days, refrigerate DNA otherwise, store in -20°C freezer.

**Extraction Protocol Two: Processing eDNA water filters using the Qiagen DNeasy Blood and Tissue Kit**

*Qiagen DNeasy Extraction kit*
*NOTE: Extraction process takes two days for isolation and all liquid waste must be disposed of in DNA buffer waste collected*

***DAY 1 - Preparations***

***BLEACH***

1. Forceps

***UV***

1. Forceps
2. Petri Dishes
3. 1.5 mL tubes
4. Tube Racks
5. 1000 µL Pipette Tips
6. 100 µL Pipette Tips
7. XL Gloves
8. L Gloves
9. Ziploc Bag

***DAY 1 – Approximately 2 hours***

1. Wipe down all equipment, including centrifuge, vortex, outside of pipettes, commonly contacted surfaces with 80% Ethanol then ELIMINase.
2. UV treat the hood that contains all the necessary equipment such as gloves, petri dishes, forceps and Kim wipes.
3. Turn on the thermomixer and set temperature to 56°C, mixing or shaking speed to 350-400 rotations per minute (rpm).
4. Take out bags with eDNA filters from freezer and keep away from UV hood.
5. Place a filter into a clean ziploc bag to serve as the extraction negative control.
6. Once UV station treatment complete, take out 2 ml tubes with Glass Beads (# of tubes = # of eDNA filters plus one extra to serve as the negative control).
7. Add **450 µL** of **Buffer ATL** to each tube *(check to see if Buffer has salt solids in bottom by opening carefully in UV station – it salt deposits are present, place in water bath for 5 minutes before pipetting).*

8. Label 2 mL tubes with sample ID and date.
9. Start with the negative control sample. Leave the filter whole, unfold the filter on the petri dish and re fold it with the eDNA side facing outwards, then place into tube.
10. Wipe down counter with ELIMINase and Ethanol between sites.
11. Place in Tissue Lyser for 2 Minutes at 30 HZ.
12. Add **40 µL** of **proteinase K** to each tube.
13. Vortex thoroughly – for 15 to 20 seconds.
14. Place the 2 mL tubes in the thermomixer at 56°C, mixing 350-400 rpm.
15. Leave overnight or anywhere between 18-24 hours.

*At this point, if you want to process another batch, you can clean up all the equipment, wipe down pipettes with a Kimwipe with ELIMINase, and UV the PCR station.*

***DAY 2 - Preparations***

***BLEACH***

1. Forceps

***UV***

7. Forceps
8. Falcon Tube for Ethanol
9. 1000 µL Pipette Tips
10. 100 µL Pipette Tips
11. 1.5 mL Tubes
12. Collection Tubes x2
13. Lo Bind Tubes
14. Tube Racks

***PREPARE***

4. DNeasy Tubes
5. Qia shredders
6. Fill UV'd Falcon tube with 100% ethanol

***DAY 2 – Approximately 3 hours***
***Set up station with DNA columns, Qia shredder columns, 1.5 mL tubes, collection tubes, forceps, ELIMINase and gloves***

1. Vortex thoroughly, 15 to 20 seconds.
2. Spin tubes down - 13,000 rpm.
3. Move each sample to a Qiashredder spin column by moving the filter with the sterilized forceps and pipette the rest of the liquid into the column.
4. Spin for two minutes at 11,000 rpm.
5. Remove liquid and place liquid into a new 1.5 mL tube.
6. Add **400 µL** of **Buffer AL** and **400 µL 100% ethanol** to each tube.
7. Vortex tubes for 15-20 seconds.
8. Spin tubes down at 13,000 rpm.

9. Label the appropriate number of DNeasy Mini Spin Column or DNA collection columns (# of DNA columns = # of eDNA filters)
10. Pipette 600 to 700 µL of the mixture that is in the 1.5 mL tube along with the precipitate into the DNeasy Mini Spin Column.
11. Centrifuge at 8,000 rpm for 1 minute.
12. Discard the flow through into the waste container and place the tube back into collection tube.
13. Pipette the remaining mixture into the spin column and repeat centrifugation and discard flow through.
14. Keep the mini spin column and place into a *new* 2 mL collection tube, and discard the used collection tube.
15. Heat Buffer AE on heating block at 70°C and set time for minimum 30 minutes without shaking.
16. Add **500 µL** of **Buffer AW1**
17. Centrifuge for 8,000 rpm for 1 minute
18. Discard the flow through into waste and discard the collection tube
19. Transfer the mini column to a *new* 2 mL collection tube
20. Add **500 µL** of **Buffer AW2.**
21. Centrifuge at 14,000 rpm for 3 minutes.
22. Discard the flow through and collection tube.
23. Keep the DNeasy Mini Spin Column.
24. Label the appropriate number of 1.5 mL Lo-Bind tubes.
25. Transfer the DNeasy Mini Spin Column into each 1.5 mL LoBind tube.
26. Pipette **110 µL** of **HOT Buffer AE** into the spin column. *Make sure you're pipetting directly onto the filter membrane.*
27. Let tubes sit at room temperature for 5 minutes.
28. Centrifuge at 8,000 rpm for 1 minute.

29. **DO NOT DISCARD THE FLOW THROUGH - IT'S THE DNA**
30. Discard the mini spin column and close the tube.

If you're going to be running an assay with DNA in the next few days, refrigerate DNA otherwise, store in -20°C or -30°C freezer.

# S3: qPCR Assay Details

Table S-1: Reaction recipe for the duplexed qPCR assay. The table displays the reaction recipe for a duplexed qPCR assay targeting the Cytochrome B gene of the invasive Sea Lamprey – from Schloesser et al. (2018) and a TaqMan™ Internal Positive Controls (IPC) that tests for PCR inhibition. The total volume per qPCR reaction is 20 µL, 5 µL of which is the DNA template. The forward primer, reverse primer, and probe are diluted to a ten nanomolar/µL working dilution before being incorporated into the reaction.

| Reagent | Volume | Product ID |
|---|---|---|
| DNA Template | 5 µl | |
| TaqMan™ Environmental Master Mix 2.0 | 10 µl | ThermoFisher Catalog Number: 4396838 |
| Forward Primer | 1 µl | See Table 1. |
| Reverse Primer | 1 µl | See Table 1. |
| Probe | 0.25 µl | See Table 1. |
| Internal Positive Control (IPC) Assay | 1.67 µl | ThermoFisher Catalog Number: 4308321 |
| Internal Positive Controls (IPC) DNA | 0.33 µl | ThermoFisher Catalog Number: 4308321 |
| Molecular Water | 0.75 µl | |

Table S-2: Cycling conditions for the duplexed qPCR assay. This table displays the cycling conditions for a qPCR TaqMan™ assay targeting the Cytochrome B gene of the invasive Sea Lamprey, duplexed with a TaqMan™ Internal Positive Controls (IPC) that tests for PCR inhibition. Assay adapted from Schloesser et al. (2018).

| Hold | 10 Minutes at 95°C |
|---|---|
| 45 Cycles | 95°C for 15 seconds,<br>60°C for 60 seconds |

Figure S-1: A qPCR standard curve of a TaqMan™ assay targeting the Cytochrome B gene of the invasive Sea Lamprey, from Schloesser et al. (2018). The standard curve was a 5-fold 10:1 serial dilution of synthetic DNA or gBlock™, through the linear dynamic range of the assay (Bustin et al. 2009). The synthetic DNA concentrations used were 7.24E+06 DNA Copies per µL down to 7.24E+02 DNA copies per µL. Eight qPCR technical replicates per dilution were used. The equation of the line was calculated to be $y = -3.4068x + 38.158$, with an $R^2 = 0.9842$. The dilution series was analyzed on the Quant Studio 7 (ThermoFisher).

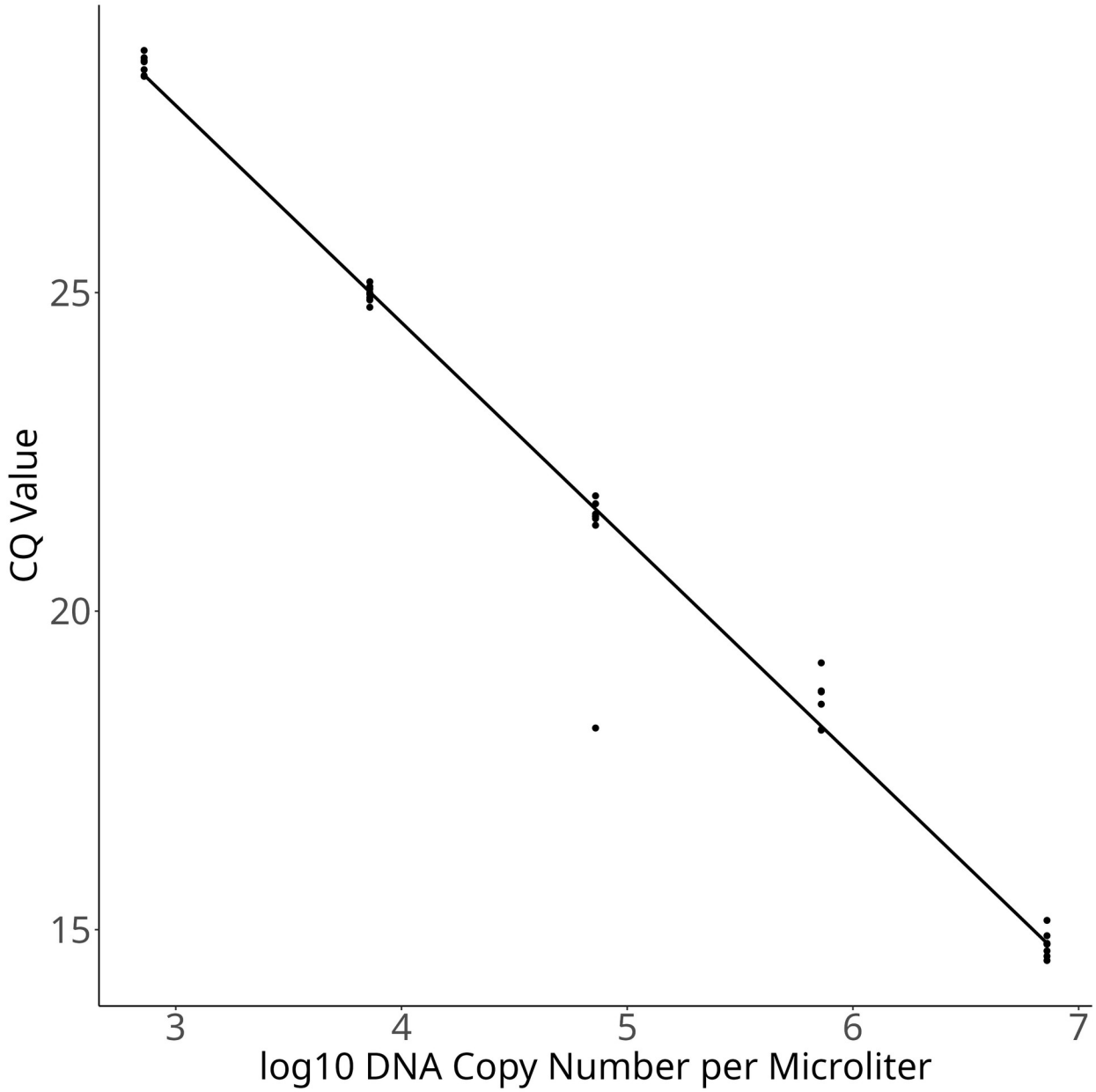


Table S-3: Table displaying the starting concentration of the synthetic DNA gBlock™ aliquoted into each qPCR replicate and the corresponding machine registered Cq values for the dilution series used to create the standard curve.

| DNA Copies per µL | Target | Reporter | Cq |
|---|---|---|---|
| 7.24E+06 | Cytb | FAM | 15.14585 |
| 7.24E+06 | Cytb | FAM | 14.77236 |
| 7.24E+06 | Cytb | FAM | 14.58366 |
| 7.24E+06 | Cytb | FAM | 14.78556 |
| 7.24E+06 | Cytb | FAM | 14.51584 |
| 7.24E+06 | Cytb | FAM | 14.77304 |
| 7.24E+06 | Cytb | FAM | 14.66658 |
| 7.24E+06 | Cytb | FAM | 14.90479 |
| 7.24E+05 | Cytb | FAM | 18.73293 |
| 7.24E+05 | Cytb | FAM | 18.13205 |
| 7.24E+05 | Cytb | FAM | Undetermined |
| 7.24E+05 | Cytb | FAM | 18.53938 |
| 7.24E+05 | Cytb | FAM | 18.13999 |
| 7.24E+05 | Cytb | FAM | 19.18825 |
| 7.24E+05 | Cytb | FAM | 18.74764 |
| 7.24E+05 | Cytb | FAM | 18.73639 |
| 7.24E+04 | Cytb | FAM | 21.34806 |
| 7.24E+04 | Cytb | FAM | 21.81 |
| 7.24E+04 | Cytb | FAM | 18.16548 |
| 7.24E+04 | Cytb | FAM | 21.49189 |
| 7.24E+04 | Cytb | FAM | 21.45195 |
| 7.24E+04 | Cytb | FAM | 21.52489 |
| 7.24E+04 | Cytb | FAM | 21.6859 |
| 7.24E+04 | Cytb | FAM | 21.48601 |
| 7.24E+03 | Cytb | FAM | 24.77081 |
| 7.24E+03 | Cytb | FAM | 24.88452 |
| 7.24E+03 | Cytb | FAM | 24.99842 |
| 7.24E+03 | Cytb | FAM | 25.09596 |
| 7.24E+03 | Cytb | FAM | 24.92078 |
| 7.24E+03 | Cytb | FAM | 25.05884 |
| 7.24E+03 | Cytb | FAM | 24.97511 |
| 7.24E+03 | Cytb | FAM | 25.17077 |
| 7.24E+02 | Cytb | FAM | 28.39515 |
| 7.24E+02 | Cytb | FAM | 28.64326 |
| 7.24E+02 | Cytb | FAM | 28.62376 |
| 7.24E+02 | Cytb | FAM | 28.80185 |
| 7.24E+02 | Cytb | FAM | 28.40661 |
| 7.24E+02 | Cytb | FAM | 28.50008 |
| 7.24E+02 | Cytb | FAM | 28.63055 |

| | | | |
|---|---|---|---|
| 7.24E+02 | Cytb | FAM | 28.68765 |
| NAC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |
| NTC | Cytb | FAM | Undetermined |

Figure S-2: Plot showing the Limit of Detection (LOD) for the duplexed Sea Lamprey Assay, with LOD values for 1, 2, 3, 4, 5, and 8 qPCR replicates per sample determined to be 19.2989171291404, 4.39348534421014, 2.52739918702137, 1.85810735133245, 1.51695913861225, and 1.06791810223353 DNA Copies per microliter of the template, respectively. The model and plot are based on Klymus et al. (2020) and were implemented in R (R Core Team).

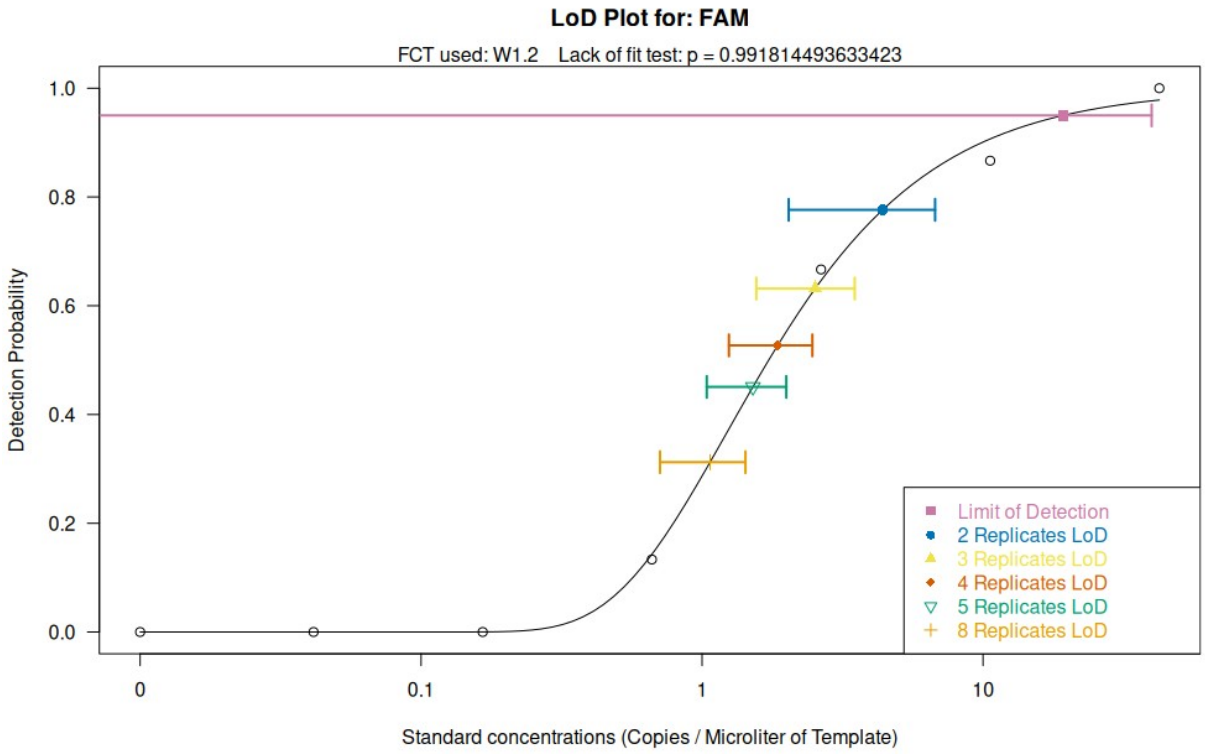
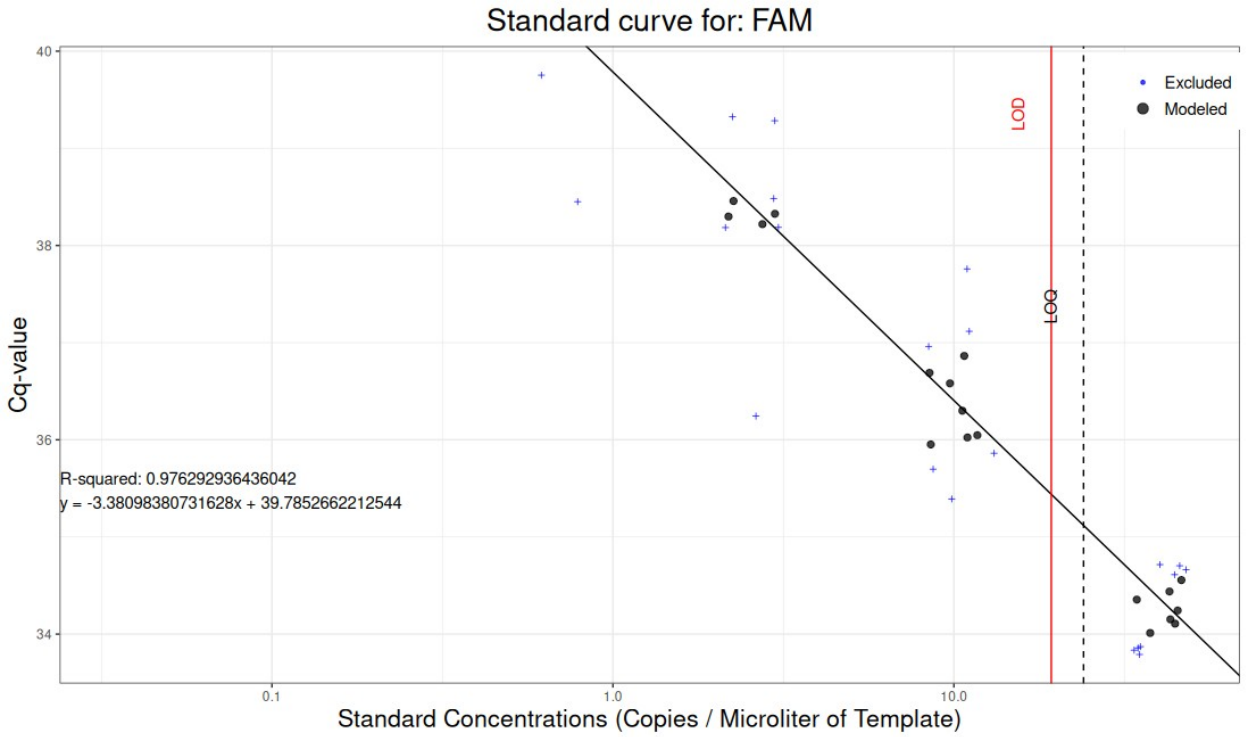


Figure S-3: Standard curve showing the relationship between Cq value and DNA copy number concentration for low concentration dilutions. It shows the Limit of Detection (LOD) – the lowest DNA concentration theoretically detectable by the qPCR assay – and the Limit of Quantification (LOQ) – the lowest DNA concentration that can be accurately quantified. The LOQ was determined to be 24 DNA Copies / µL of template, using a CV threshold of 0.35. Estimates are for a qPCR assay from Schloesser et al. (2018) that targets the Cytochrome B gene of the invasive Sea Lamprey. Plot and model are from Klymus et al. 2020 and were implemented in R (R Core Team).



# S4: Sampling Station Coordinates for the Filtration Device Comparison

Table S-4: Coordinates for the 28 Sampling Stations surveyed for Experiment 2. “Sampling Station” is the ID for the location at which eDNA samples were collected for the filtration device comparison in Experiment 2.

| Sampling Station | Latitude | Longitude |
|---|---|---|
| GLFC_AU-003-F | 46.37695 | -86.84307 |
| GLFC_BR-001-F | 43.439534 | -79.87234 |
| GLFC_BR-002-F | 43.440859 | -79.885044 |
| GLFC_BR-003-F | 43.452519 | -79.920734 |
| GLFC_BR-004-F | 43.414912 | -79.9288 |
| GLFC_BR-005-F | 43.407697 | -79.943352 |
| GLFC_CD-005-F | 45.666776 | -87.399201 |
| GLFC_CR-001-F | 43.581742 | -79.707338 |
| GLFC_CR-002-F | 43.602544 | -79.718249 |
| GLFC_CR-003-F | 43.632625 | -79.75814 |
| GLFC_CR-004-F | 43.644508 | -79.79864 |
| GLFC_CR-005-F | 43.64948 | -79.858544 |
| GLFC_ET-003-F | 44.260995 | -87.660291 |
| GLFC_ET-005-F | 44.236858 | -87.637446 |
| GLFC_GL-001-F | 46.71377641 | -84.28956105 |
| GLFC_GL-002-F | 46.74646001 | -84.117548 |
| GLFC_GL-003-F | 46.7809759 | -84.03384936 |
| GLFC_HR-001-F | 46.84496849 | -84.37340289 |
| GLFC_HR-004-F | 46.84875853 | -84.36641009 |
| GLFC_HR-005-F | 46.84911163 | -84.35635796 |
| GLFC_LY-001-F | 43.875943 | -78.960707 |
| GLFC_LY-002-F | 43.892999 | -78.968966 |
| GLFC_LY-003-F | 43.880529 | -78.984997 |
| GLFC_LY-004-F | 43.926854 | -78.965585 |
| GLFC_LY-005-F | 43.96125 | -79.002988 |
| GLFC_PN-001-F | 46.96633143 | -84.6592801 |
| GLFC_PN-002-F | 46.97682086 | -84.67720379 |
| GLFC_PN-003-F | 46.9918393 | -84.68112979 |

# S5: Analysis of Cq mean and variance by run for the filtration device comparison experiment.

Figure S-4: Analysis of inter-plate variation for Experiment 2. Graph showing the range of Cq values for three 96-well qPCR plates used to analyze environmental samples for invasive Sea Lamprey DNA. ANOVA test on the range of Cq values by plates showed no significant difference in the mean value Cq value between the three plates (P=0.2993, df=2) .

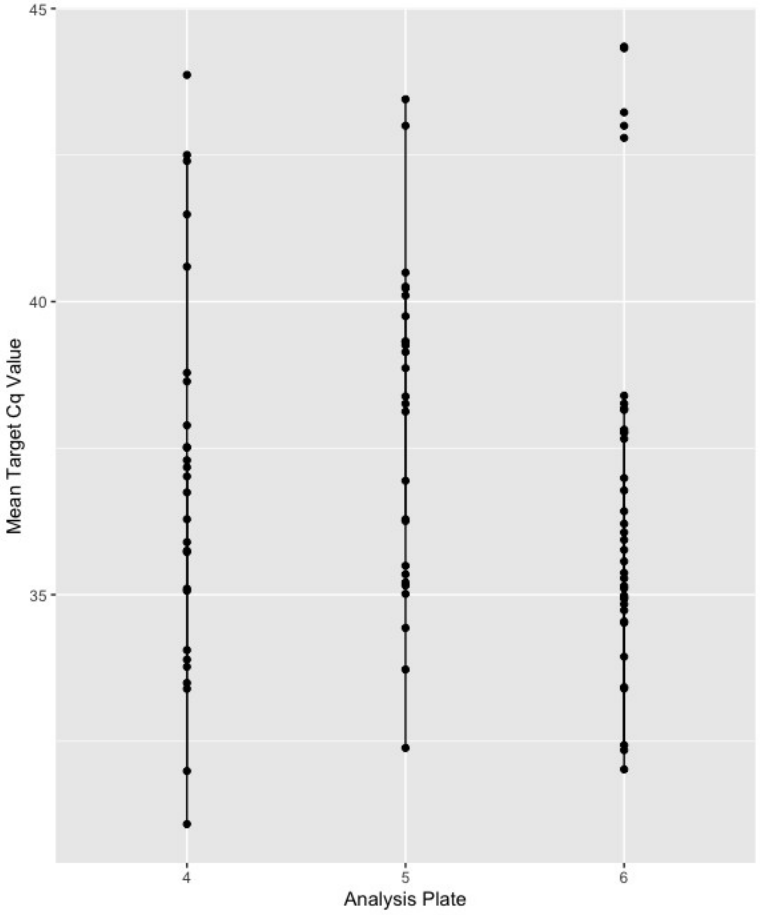
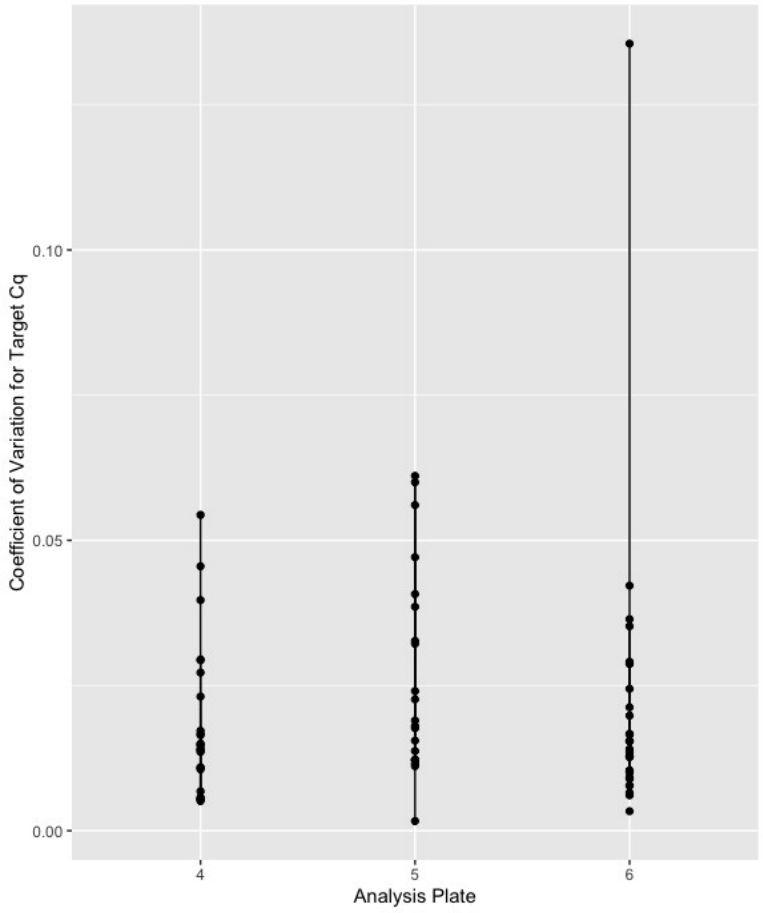


Figure S-5: Analysis of the difference in Coefficient of Variation (CV) of Cq values for qPCR plates analyzed for Experiment 2. Graph showing the Coefficient of Variation (CV) of the Cq values for three 96-well qPCR plates used to analyze environmental samples for invasive Sea Lamprey DNA. ANOVA test on the range of Cq values by plates showed no significant difference in the CV of Cq values between the three plates (P=0.2992, df=2).



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