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

Cameron D. Brown[1], Robert H. Hanner[1], and Margaret F. Docker[2]

[1]Department of Integrative Biology, University of Guelph

[2]Department of Biological Sciences, University of Manitoba

September 13, 2024

The publisher is not responsible for the content or functionality of any supporting information supplied by the authors. Any queries should be directed to the corresponding author for the article.

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 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 Matches code on coin envelope. 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, 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).

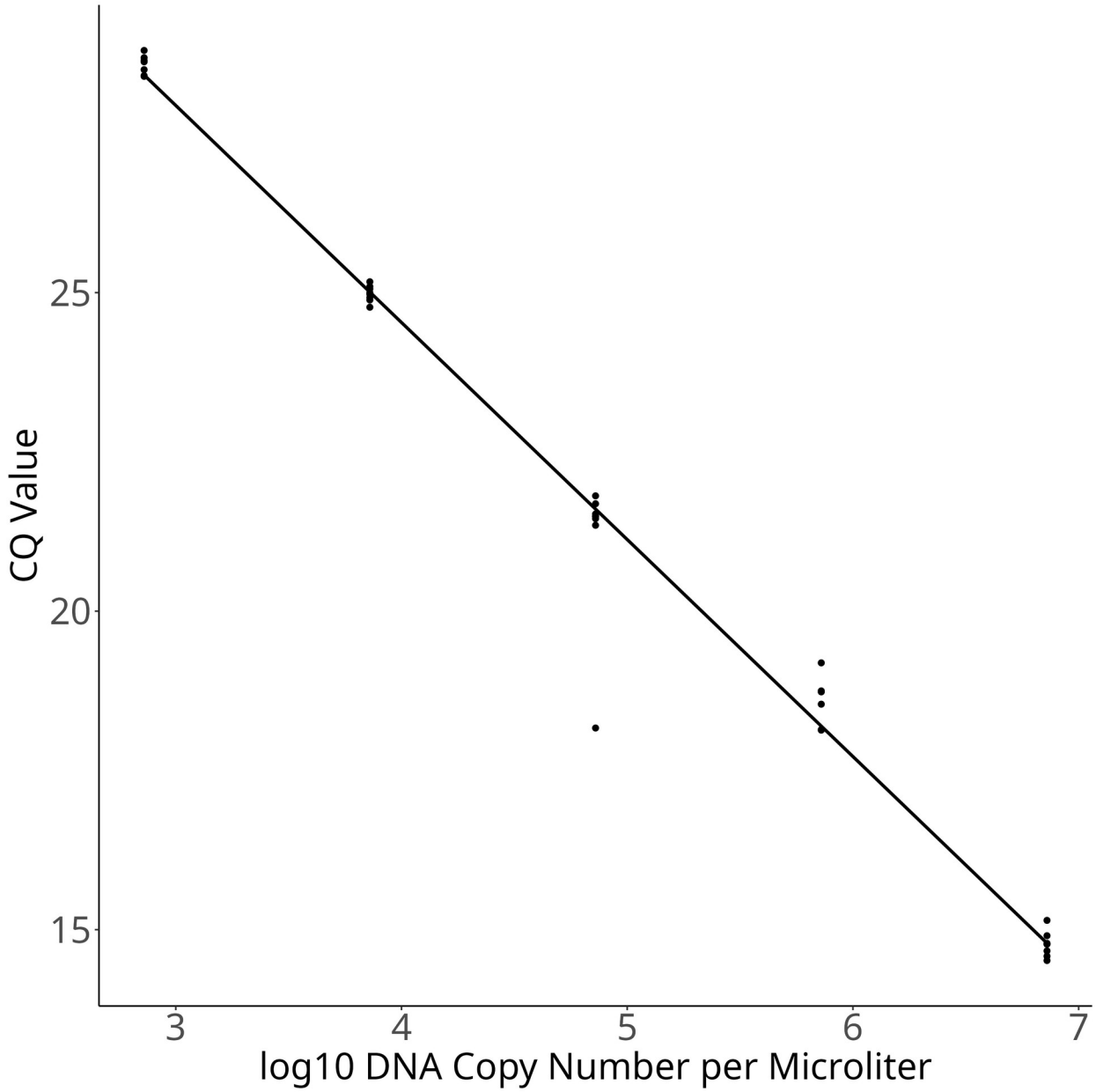


Table S-3: Table displaying the starting concentration of the synthetic DNA gBlock™ aliquoted into each qPCR replicate and the corresponding machine registered Cq values for the dilution series used to create the standard curve.

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).

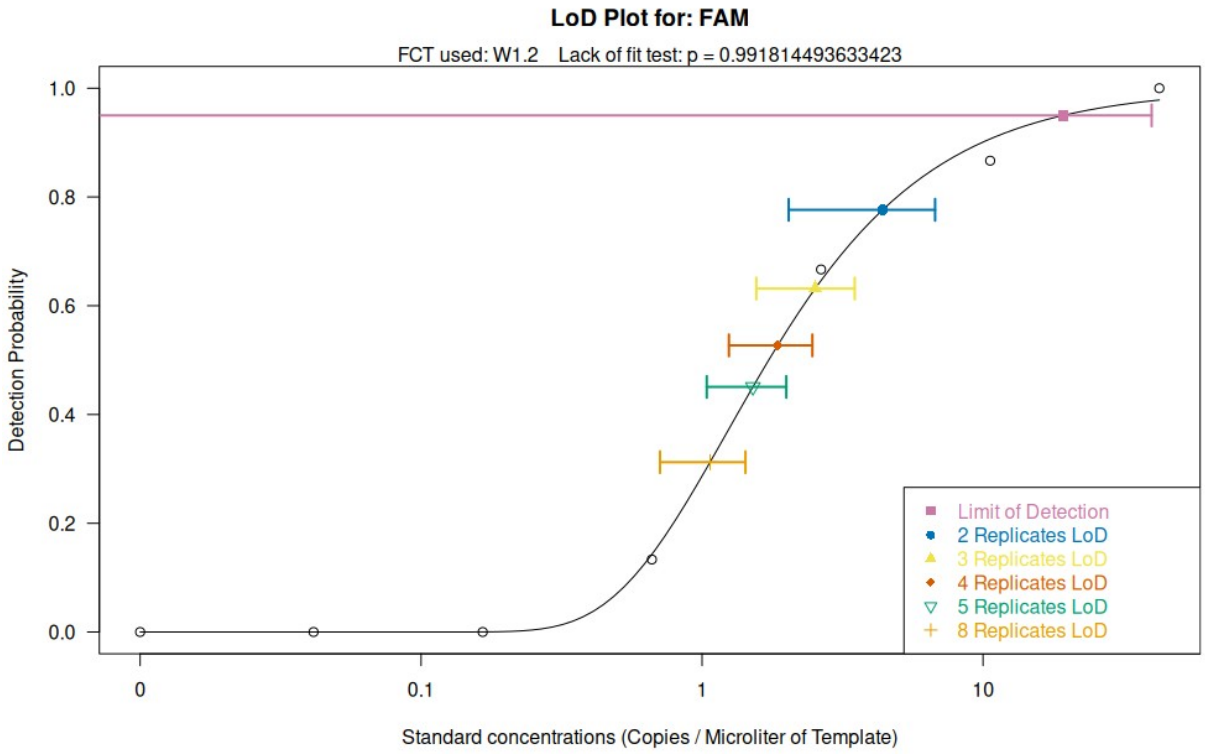
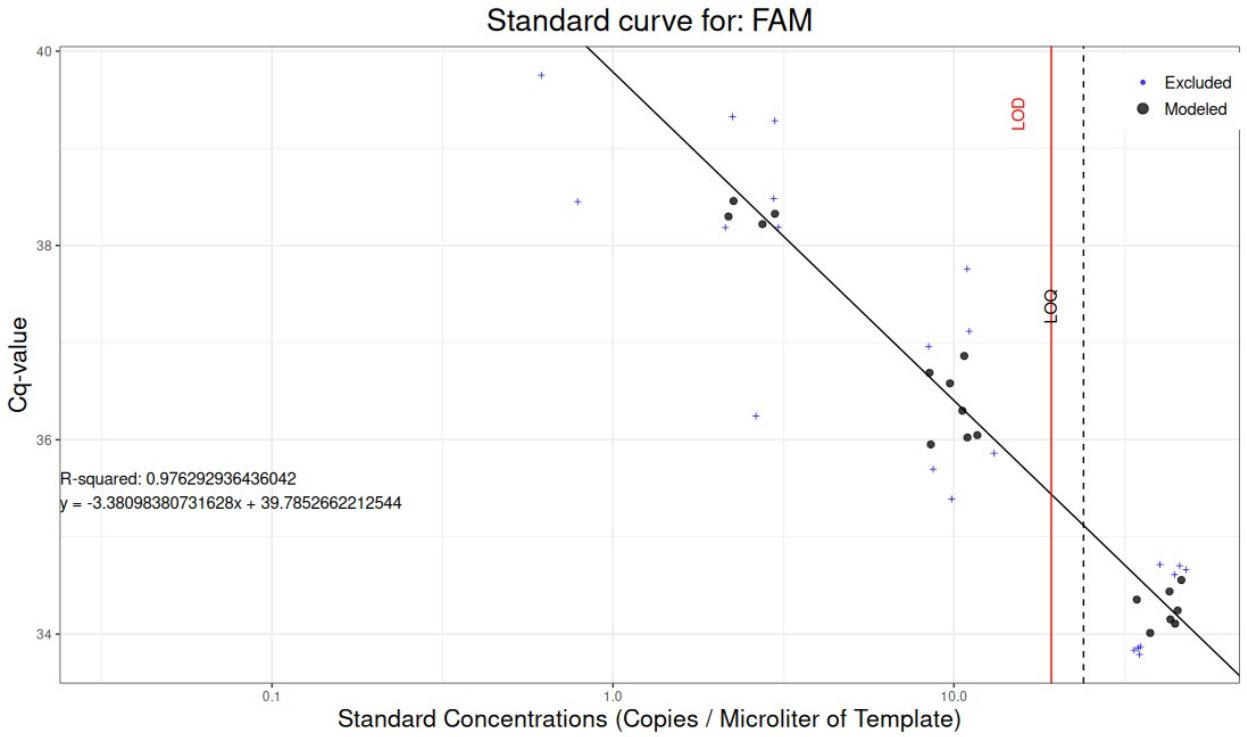


Figure S-3: Standard curve showing the relationship between Cq value and DNA copy number concentration for low concentration dilutions. It shows the Limit of Detection (LOD) – the lowest DNA concentration theoretically detectable by the qPCR assay – and the Limit of Quantification (LOQ) – the lowest 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) .

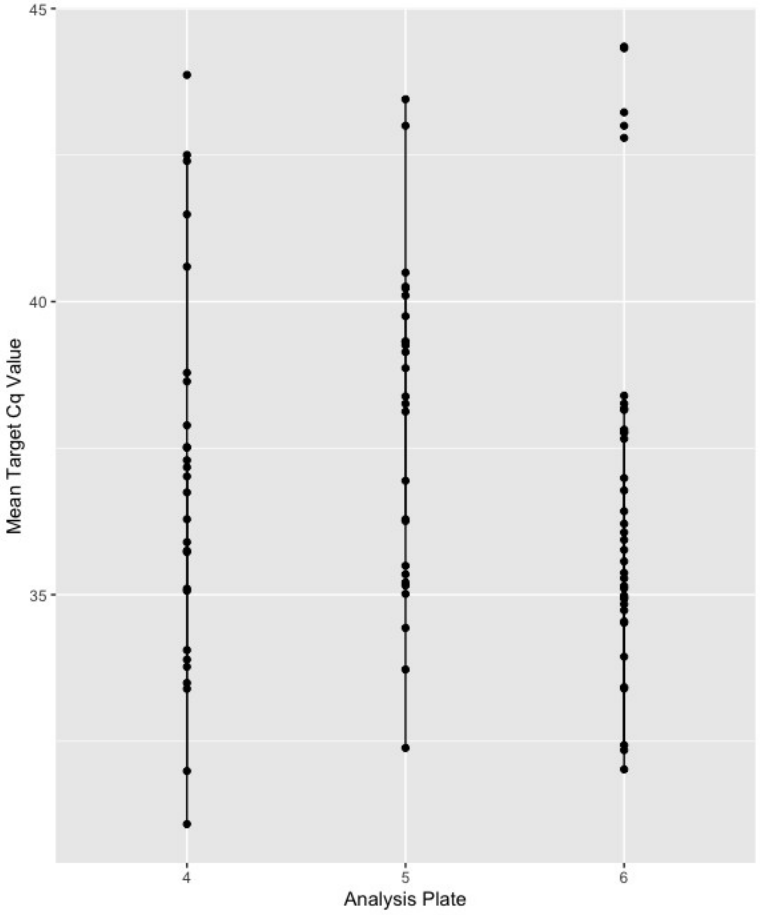
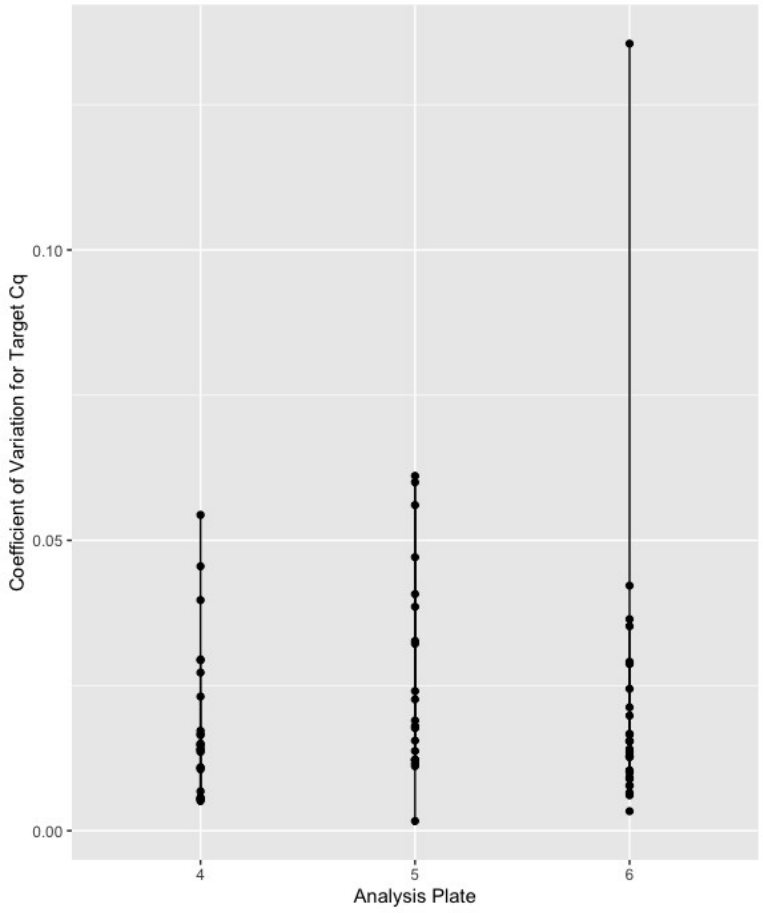


Figure S-5: Analysis of the difference in Coefficient of Variation (CV) of Cq values for qPCR plates analyzed for Experiment 2. Graph showing the Coefficient of Variation (CV) of the Cq values for three 96-well qPCR plates used to analyze environmental samples for invasive Sea Lamprey DNA. ANOVA test 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).



References

Bustin, S. A., V. Benes, J. A. Garson, J. Hellemans, J. Huggett, M. Kubista, R. Mueller, T. Nolan, M. W. Pfaffl, G. L. Shipley, J. Vandesompele, and C. T. Wittwer. 2009. The MIQE Guidelines: Minimum information for publication of quantitative real-time PCR experiments. Clinical Chemistry 55(4):611–622.

Klymus, K. E., C. M. Merkes, M. J. Allison, C. S. Goldberg, C. C. Helbing, M. E. Hunter, C. A. Jackson, R. F. Lance, A. M. Mangan, E. M. Monroe, A. J. Piaggio, J. P. Stokdyk, C. C. Wilson, and C. A. Richter. 2020. Reporting the limits of detection and quantification for environmental DNA assays. Environmental DNA 2(3):271–282.

Schloesser, N. A., C. M. Merkes, C. B. Rees, J. J. Amberg, T. B. Steeves, and M. F. Docker. 2018. Correlating sea lamprey density with environmental DNA detections in the lab. Management of Biological Invasions 9(4):483–495.