

# **Invasive Species Detection in Northeast Regional Lakes using Environmental DNA (eDNA) - Zebra Mussel and Asian Clam Pilot Study 2019**

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### **Introduction**

The objective of this study was to develop and conduct a pilot environmental DNA (eDNA) sampling program for target invasive species in northeastern lakes. This project provides proof of concept and initial eDNA data for two invasive invertebrates; asian clam (*Corbicula fluminea*) and zebra mussel (*Dreissena polymorpha*). Both of these species are present in some areas of the region, and are considered at risk of invading currently uninfected lakes. Improved early detection methods will support timely response and management actions.

eDNA is genetic material in an environmental sample, such as water or sediment. It may contain entire microorganisms (like algae or larvae) or fragments of tissue, reproductive and waste products, and other cellular material from larger organisms. eDNA is emerging as a method for early detection of invasive species in lakes and other waterbodies, but requires species-specific validation to inform management objectives.

### **Approach**

The work was conducted by the University of New Hampshire in collaboration with State Agencies and other stakeholders in Maine, New Hampshire, Vermont, and New York. Water samples were collected in lakes with known infestations of zebra mussels and/or asian clam. Initially, we sampled two lakes where both species were present (Lake Bomoseen, VT and Lake George, NY), and one control site where neither species is expected to be present (Toddy Pond, ME). Sites were sampled in the spring, summer and fall to capture seasonal changes. Additional sites in Lake George, Lake Memphremagog VT, Lake Dunmore VT, and Great Pond NH, were added over the course of the summer to provide data on newly identified zebra mussel occurrences, and increase spatial coverage for asian clam.

### **Methods**

*Sample collection:* Five 1-liter water samples were collected at each sampling location following the protocol in Appendix B. Briefly, samples were filtered onsite through 1.5 um glass fiber filters, then frozen until shipped to UNH for analysis. All sampling equipment, including waders, that contacted the waterbody was either new or decontaminated between sites. All samples were collected from slightly below the water surface, and accessed by wading or dipping from a dock.

*Extraction and analysis:* Filters were cut in half, and one half was extracted for analysis, while the remaining half was retained for storage. Extractions were performed on a QiaCube Connect system using a modified version of the QIAGEN Buccal Swab 400 Protocol. We have found this to be equivalent to the QIAGEN Blood and Tissue protocol modified for the QiaCube system, with lyse and spin baskets to facilitate extraction from filters.

Analysis was performed on a Biorad Digital Droplet PCR system (ddPCR) using species specific assays. Initially, zebra mussel analyses were conducted with an assay described in Amberg et al. (2019), but after review of results and further discussion with personnel at the USGS a *Dreissena* ssp. assay (DRE16S, Gingera et al. 2017) was adopted. This analysis does not distinguish between zebra mussels and quagga mussels, but may have lower detection limits than other published assays (Supulveda et al. 2019). A published asian clam assay (Cowart et al. 2018) was modified to amplify a wider range of species variants. Details of the PCR parameters, reagents, and assays are included in Appendix C.

*Quantification and detection limits:* The ddPCR method divides the sample aliquot into micro droplets where each droplet is amplified separately to create a 'count' of positive reactions. Droplets with amplified target sequences fluoresce, and the amplitude of the fluorescence is used to separate positive and negative reactions. In rare cases reflection or other optical interference may result in a false positive reading, and samples with less than 3 positive droplets should be treated with caution. Results are reported as positive counts (number of droplets where the target sequence amplifies), and as the concentration of amplified sequence copies in the sample extract. Samples with 1 or 2 positive droplets have been included in the average concentration for each site, but are flagged L (low), and are not included as detections in the positive sample count.

### **Quality Assurance Quality Control**

One of the more difficult aspects of eDNA sampling programs is avoiding cross contamination between sites or in the lab. We address this by a) following procedures outlined in the field protocol for decontaminating between sites, and in all stages of sample handling, and b) generating field and laboratory blanks to identify potential contamination throughout the process. Trip blanks, consisting of clean lab water, accompanied all samples. Filter blanks, extraction blanks, no template controls, and positive and negative PCR controls were generated and run with each sample batch as summarized below:

- A field blank (a clean filter, exposed at the field site, then stored and shipped with field samples) was collected at each sampling location.
- Extraction blanks (a clean filter extracted with the samples) was included in each extraction run (up to 12 samples per run).
- Positive and negative PCR controls were included in each ddPCR analysis run. Positive controls were dilute tissue extractions from each species. Negative controls were ultrapure water.

### **Sample sites and results**

#### ***Lake Bomoseen, VT***

Samples were collected at three locations in Lake Bomoseen VT. Viable populations of both zebra mussel and Asian clam are present in the southern region of the lake. BOM1 is in the northern section of the lake, approximately 3 miles from the known infestation, BOM2 is on the western shore, between sites 1 and 3, and BOM3 is on the southwest shore near the infestation. The sites were sampled in May, July and October (Table 1). Zebra mussels were detected at all locations in May and July, with the highest concentrations of target DNA in samples in May. Both the concentration and number of positive samples were lowest in October.

**Table 1. Zebra mussel detections in Lake Bomoseen, VT.** Summary of 5 1-liter samples collected at each site. Full data table in Appendix A

Site	Date Collected	Water Temp °C	Mean concentration (copies/μL)	Number of positive samples
BOM1	5/28/2019	11	9.15	5/5
BOM1	7/8/2019	25.5	1.19	3/5
BOM1	10/21/2019	14*	0.03	0/5
BOM2	5/28/2019	12	394.89	5/5
BOM2	7/8/2019	24.5	220.60	5/5
BOM2	10/21/2019	14*	1.67	4/5
BOM3	5/28/2019	6	389.70	5/5
BOM3	7/8/2019	24.5	315.69	5/5
BOM3	10/21/2019	14*	1.03	3/5

\*Temperature data not collected on 10/21/19 sampling date. Temp estimated from nearby USGS gauge.

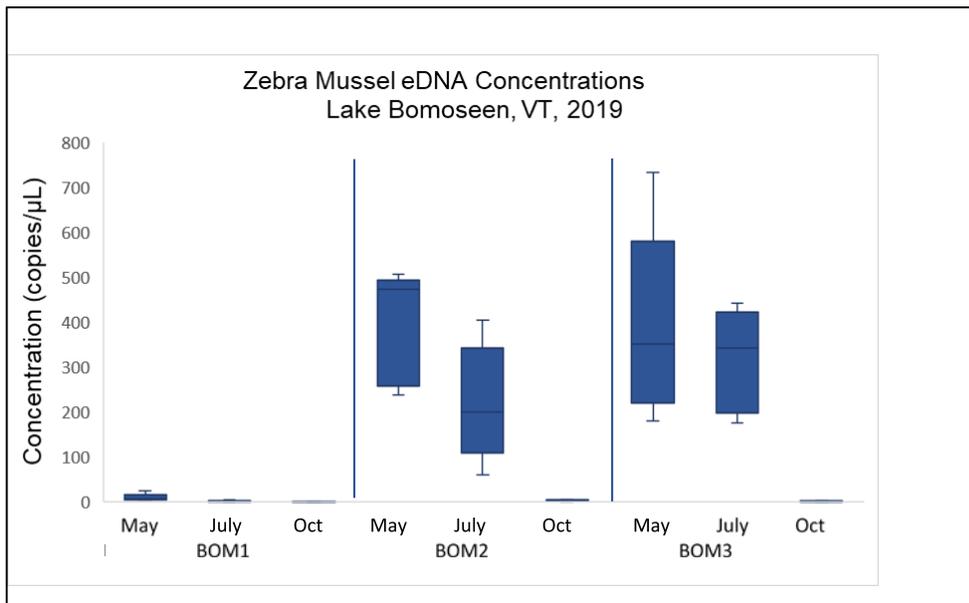
Asian clam was detected in samples from all three sites in May and July, but was present at low levels only in samples from BOM2 and BOM3 in October (table 2).

**Table 2. Asian clam detections in Lake Bomoseen, VT.** Summary of 5 1-liter samples collected at each site. Full data table in Appendix A.

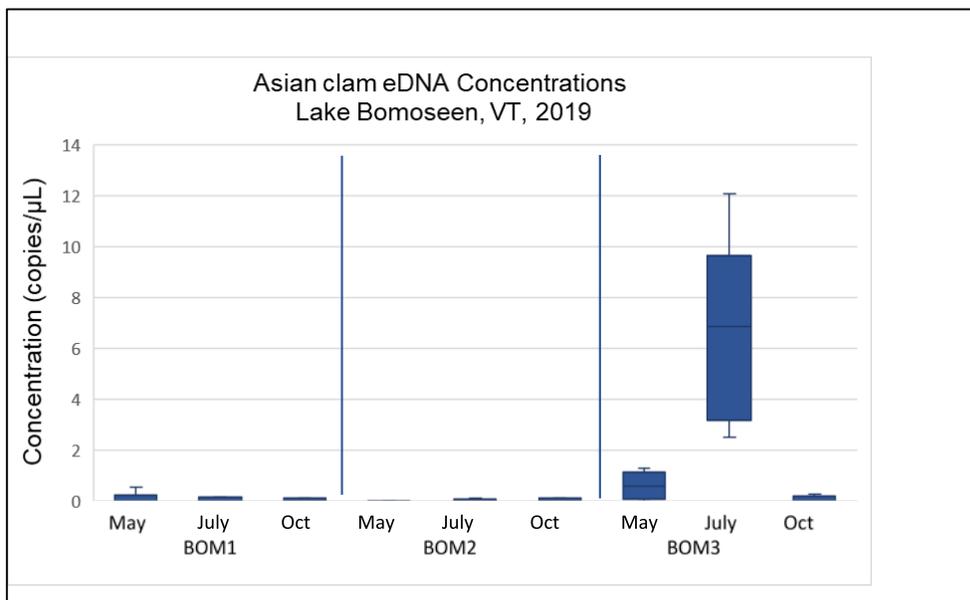
Site	Date Collected	Water Temp °C	Mean concentration (copies/μL)	Number of positive samples
BOM1	5/28/2019	11	0.10*	1/5
BOM1	7/8/2019	25.5	0.05	0/5
BOM1	10/21/2019	14*	0.05	0/5
BOM2	5/28/2019	12	0.00	0/5
BOM2	7/8/2019	24.5	0.03	0/5
BOM2	10/21/2019	14*	0.04*	0/5
BOM3	5/28/2019	6	0.59	3/5
BOM3	7/8/2019	24.5	6.50	5/5
BOM3	10/21/2019	14*	0.08	3/5

\*1 droplet detected in site trip blank

Figures 1 and 2 show the concentrations of both species in Lake Bomoseen. Concentrations are highest in the spring and summer; with very low to no detections in October.



**Figure 1.** Zebra mussel eDNA concentrations in Lake Bomoseen. Five 1-liter samples were collected at each location. Concentrations are highest in early spring, suggesting that spawning occurs while lake waters are still below 12C.



**Figure 2.** Asian clam eDNA concentrations in Lake Bomoseen. Five 1-liter samples were collected at each location. Concentrations are highest in spring/summer, and the signal is strongest near the source.

**Lake George, NY**

Asian clam colonies are present in the southern sections of the lake near sites LG3, LG4 and LG5. Zebra mussel colonies have also been found in this region, but low calcium concentrations appear to inhibit reproduction and spread of zebra mussel in Lake George. When zebra mussels are found in Lake George they are removed manually by divers. In 2019, zebra mussels were documented in low abundance (~20 individuals) with adults only at LG4 and LG5 and at LG3 adult and juvenile mussels were documented in the fall. Samples were collected at five locations in Lake George (NY). Samples were initially collected at LG1 near Huletts Landing, LG2 at Pilot Knob Beach, and LG3 at Million Dollar Beach. No zebra mussel was initially detected in any of these samples, and two additional sites, LG4 at Rogers Rock Campground, and LG5 at Beckleys Marina were sampled in September. All samples were re-run using the DRE16S assay, and zebra mussel was detected at very low levels in samples collected at LG3 in July.

**Table 3. Zebra mussel detections in Lake George, NY.** Summary of 5 1-liter samples collected at each site. Full data table in Appendix A

Site	Date Collected	Water Temp °C	Mean concentration (copies/μL)	Number of positive samples
LG1	5/28/2019	12	0.00	0/5
LG1	7/8/2019	21	0.00	0/5
LG2	5/28/2019	12	0.00	0/5
LG2	7/8/2019	23	0.00	0/5
LG3	5/28/2019	12	0.00	0/5
LG3	7/8/2019	23	0.14	1/5
LG4	9/24/2019	19	0.00	0/5
LG5	9/24/2019	19	0.00	0/5

These samples were rerun (with the DRE16S assay), and zebra mussel was again detected at LG3 in the July samples. However, all of concentrations were very low; four of the analyses contained less than three droplets and should be considered tentative detections.

**Table 4. Asian clam detections in Lake George, NY.** Summary of samples collected at each site. Full data table in Appendix A.

Site	Date Collected	Water Temp °C	Mean concentration (copies/μL)	Number of positive samples
LG1	5/28/2019	12	0.00	0/5
LG1	7/8/2019	21	0.00	0/5
LG2	5/28/2019	12	0.00	0/5
LG2	7/8/2019	23	0.00	0/5
LG3	5/28/2019	12.5	0.26	2/5
LG3	7/8/2019	24.5	0.41	3/5
LG4	9/24/2019	19	1.74	5/5
LG5	9/24/2019	19	1.63	5/5

Asian clam was detected at sites LG3, LG4 and LG5. Clam shells were noted on the field forms at LG5.

**Lake Memphremagog, VT**

Lake Memphremagog stretches about 50km from Newport, VT to Magog, QC, Canada. A new colony of Zebra mussels was identified in 2019 in the northern section of Lake Memphremagog in Magog Bay. eDNA samples were collected at 5 locations; MG1 at Pointe Merry near the site of the observed infestation, MG3 at Georgeville, MG4 at Cedarville in Canada, and MG5 at the Newport City dock in VT. MG2, at the Magog municipal water pump, was inaccessible and not sampled. Very low (<3 droplets) concentrations were detected in one (of five) samples from MG1, MG3 and MG5, and the trip blank from MG1. These results should be considered tentative, and potentially compromised by field contamination. Asian clam analyses was not conducted on these samples.

**Table 5. Zebra mussel detections in Lake Memphremagog, QC/VT.** Summary of 5 1-liter samples collected at each site. Full data table in Appendix A.

Site	Date Collected	Water Temp °C	Mean concentration (copies/µL)	Number of positive samples
MG1	8/23/2019	17.2	0.05*	0/5
MG3	8/23/2019	18.9	0.03	0/5
MG4	8/23/2019	18.9	0.00	0/5
MG5	8/23/2019	18.3	0.02	0/5

\*1 droplet detected in MG1 trip blank

**Lake Dunmore, VT**

Several hundred zebra mussels were found in Lake Dunmore, VT in late summer 2019. All of the observed mussels were removed in September, 2019. eDNA water samples were collected on October 16, 2019. To maximize probability of detection, larger (3 liter) volumes were collected. Neither Zebra mussel or Asian clam were detected in the water samples collected at this site.

**Great Pond, NH**

Asian clam are present near a boat ramp in Great Pond, NH. Samples were collected at the boat ramp GP2, and at Kingston State Park (GP1). Asian clam was detected in all of the samples collected at GP1, near the colony, and were not detected at GP2, less than 1km across the lake.

**Table 6. Asian clam detections in Great Pond, NH.** Summary of samples collected at each site. Full data table in Appendix A.

Site	Date Collected	Water Temp °C	Mean concentration (copies/µL)	Number of positive samples
GP1	8/22/2019	18	0.00	0/1
GP2	8/22/2019	18	1.03	5/4

**Toddy Pond, ME**

Samples were collected at Toddy Pond in Maine in May and July. Neither zebra mussel or Asian clam are expected to be present, and neither were detected in samples.

### **Quality Assurance Quality Control**

All extraction blanks and negative PCR controls were negative (no detections). Very low (one droplet) detections of asian clam were found in three trip blanks; BOM1 5/28, BOM2 10/21, and MG1 8/23.

### **Summary and recommendations**

We collected and analyzed samples from 16 sites in six New England lakes from May to October, 2019. All samples were analyzed by ddPCR for Asian clam DNA, Zebra mussel DNA or both.

- Asian clam analysis successfully detected DNA near known infestations in three lakes, with the concentration decreasing with distance.
- Zebra mussel analysis detected DNA in a lake with a viable population (Lake Bomoseen), but detected very low or no signal at sites with smaller infestations (Lake George and Lake Memphremagog), or where an infestation had been recently removed (Lake Dunmore). Greater sensitivity might be obtained with larger sample volumes and/or higher sample number.

Concentrations were higher in spring/early summer.

- Zebra mussel signal in Lake Bomoseen was highest in May, slightly lower in July, and much lower in October. Sampling in early spring will likely improve detection probability.
- Asian clam concentration was highest in July and May, and lowest in October. The Asian clam signal may peak slightly later in the spring than zebra mussel. Additional analysis will support the development of occupancy models to estimate sampling design detection probabilities.

### **Recommendations**

- Re-sample low concentration sites (Lake George, Memphremagog, possibly Lake Dunmore) in early spring, taking larger volume samples, and focusing on areas with known colonies to develop low concentration detection methods.

### **Acknowledgements**

This study was funded by the Vermont Department of Environmental Conservation, the New Hampshire Department of Environmental Services and the Maine Department of Environmental Protection. Staff from these agencies, and from the New York Department of Environmental Conservation and the Lake Champlain Basin Program collected samples, provided feedback on methods and information on site biology, and assisted in interpretation of results. Adam Sepulveda, Richard Erickson and others at the USGS provided advice on assay selection and sampling design. Samples were analyzed at the Hubbard Center for Genome Studies at the University of New Hampshire with advice from HCGS director Kelly Thomas, and HCGS staff Jeffery Hall and Steve Simpson.

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## APPENDICES

### APPENDIX A – Data summary

Tables summarizing data from sites with positive results. Sites with no data listed here had no positive results for either species

### APPENDIX B – Environmental DNA (eDNA) Sampling Protocol

### APPENDIX C – Analytical Methods and Species Specific Assays

### APPENDIX D – Field data sheets

## **APPENDIX A – Data Summary**

Tables summarizing data from sites with positive results. Sites with no data listed here had no positive results for either species

Lake Bomoseen Zebra Mussel

Lake Bomoseen Zebra Mussel Run Comparison

Lake Bomoseen Asian Clam

Lake George Zebra Mussel

Lake George Asian Clam

Lake Memphremagog Zebra Mussel

Great Pond Asian Clam

There were no positive detections in samples collected Lake Dunmore, VT, and a data summary is not included here.

Lake Bomoseen - Zebra Mussel					
Sample ID	Site	Date Collected	Water Temp °C	Conc(copies/µL)	Positive droplets
BOM1w0528191	BOM1	5/28/2019	11	4.16	29
BOM1w0528192	BOM1	5/28/2019	11	5.38	36
BOM1w0528193	BOM1	5/28/2019	11	23.28	174
BOM1w0528194	BOM1	5/28/2019	11	4.41	28
BOM1w0528195	BOM1	5/28/2019	11	8.51	60
BOM1tb0528195	tb	5/28/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>9.15</b>	<b>5/5</b>
BOM1w0708191	BOM1	7/8/2019	25.5	0.24	2 L
BOM1w0708192	BOM1	7/8/2019	25.5	0.00	0
BOM1w0708193	BOM1	7/8/2019	25.5	0.96	6
BOM1w0708194	BOM1	7/8/2019	25.5	0.81	7
BOM1w0708195	BOM1	7/8/2019	25.5	3.92	30
BOM1tb0708195	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>1.19</b>	<b>3/5</b>
BOM1w1021191	BOM1	10/21/2019	---	0.00	0
BOM1w1021192	BOM1	10/21/2019	---	0.00	0
BOM1w1021193	BOM1	10/21/2019	---	0.14	1 L
BOM1w1021194	BOM1	10/21/2019	---	0.00	0
BOM1w1021195	BOM1	10/21/2019	---	0.00	0
BOM1tb1021195	tb	10/21/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.03</b>	<b>0/5</b>
BOM2w0528191	BOM2	5/28/2019	12	279.19	1592
BOM2w0528192	BOM2	5/28/2019	12	507.00	3012
BOM2w0528193	BOM2	5/28/2019	12	478.17	3074
BOM2w0528194	BOM2	5/28/2019	12	236.91	1384
BOM2w0528195	BOM2	5/28/2019	12	473.17	2626
BOM2tb0528195	tb	5/28/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>394.89</b>	<b>5/5</b>
BOM2w0708191	BOM2	7/8/2019	24.5	60.21	360
BOM2w0708192	BOM2	7/8/2019	24.5	158.13	902
BOM2w0708193	BOM2	7/8/2019	24.5	282.15	1927
BOM2w0708194	BOM2	7/8/2019	24.5	199.29	1337
BOM2w0708195	BOM2	7/8/2019	24.5	403.22	2277
BOM2tb0708195	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>220.60</b>	<b>5/5</b>
BOM2w1021191	BOM2	10/21/2019	---	1.14	8
BOM2w1021192	BOM2	10/21/2019	---	1.65	10
BOM2w1021193	BOM2	10/21/2019	---	0.00	0

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake Bomoseen - Zebra Mussel					
Sample ID	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
BOM2w1021194	BOM2	10/21/2019	---	1.13	8
BOM2w1021195	BOM2	10/21/2019	---	4.44	33
BOM2tb1021195	tb	10/21/2019	---	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>1.67</b>	<b>4/5</b>
BOM3w0528191	BOM3	5/28/2019	6	260.06	1654
BOM3w0528192	BOM3	5/28/2019	6	733.80	3331
BOM3w0528193	BOM3	5/28/2019	6	178.72	1154
BOM3w0528194	BOM3	5/28/2019	6	426.01	2297
BOM3w0528195	BOM3	5/28/2019	6	349.89	2650
BOM3tb0528195	tb	5/28/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>389.70</b>	<b>5/5</b>
BOM3w0708191	BOM3	7/8/2019	24.5	401.90	2653
BOM3w0708192	BOM3	7/8/2019	24.5	341.60	2450
BOM3w0708193	BOM3	7/8/2019	24.5	175.99	1388
BOM3w0708194	BOM3	7/8/2019	24.5	442.00	2695
BOM3w0708195	BOM3	7/8/2019	24.5	216.96	1335
BOM3tb0708195	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>315.69</b>	<b>5/5</b>
BOM3w1021191	BOM3	10/21/2019	---	2.04	13
BOM3w1021192	BOM3	10/21/2019	---	0.41	3
BOM3w1021193	BOM3	10/21/2019	---	0.64	5
BOM3w1021194	BOM3	10/21/2019	---	0.21	1 L
BOM3w1021195	BOM3	10/21/2019	---	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>1.03</b>	<b>3/5</b>
NTC				0.00	0
ZM 1 : 100				1,687.86	6355
ZM 1 : 1,000				35.16	243
No tb for BOM102119					

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake Bomoseen - Zebra Mussel Run Comparison				
Sample ID	Conc(copies/ $\mu$ L)		Positive droplets	
	Run A	Run B	Run A	Run B
BOM1w1021191	0.00	0.00	0	0
BOM1w1021192	0.00	0.00	0	0
BOM1w1021193	0.14	0.00	1 L	0
BOM1w1021194	0.00	0.21	0	2 L
BOM1w1021195	0.00	0.00	0	0
BOM1tb102119	0.00	0.00	0	0
<b>Average concentration</b>	<b>0.03</b>	<b>0.04</b>		
BOM2w1021191	1.14	1.29	8	6
BOM2w1021192	1.65	1.11	10	4
BOM2w1021193	0.00	0.00	0	0
BOM2w1021194	1.13	0.76	8	3
BOM2w1021195	4.44	3.13	33	14
BOM2tb102119	0.00	0.00	0	0
<b>Average concentration</b>	<b>1.67</b>	<b>1.26</b>		
BOM3w1021191	2.04	0.35	13	1 L
BOM3w1021192	0.41	0.00	3	0
BOM3w1021193	0.64	0.58	5	3
BOM3w1021194	0.21	0.27	1 L	2 L
BOM3w1021195	0.00	0.00	0	0
<b>Average concentration</b>	<b>0.66</b>	<b>0.24</b>		
ZM 1 : 1,000	35.16	44.46		

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake Bomoseen - Asian Clam					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
BOM1w0528191	BOM1	5/28/2019	11	0.00	0
BOM1w0528192	BOM1	5/28/2019	11	0.00	0
BOM1w0528193	BOM1	5/28/2019	11	0.00	0
BOM1w0528194	BOM1	5/28/2019	11	0.00	0
BOM1w0528195	BOM1	5/28/2019	11	0.52	3
BOM1wTB052819	tb	5/28/2019		0.14	1 L
<b>Average concentration &amp; number of positive samples</b>				<b>0.10</b>	<b>1/5</b>
BOM1w0708191	BOM1	7/8/2019	25.5	0.13	1 L
BOM1w0708192	BOM1	7/8/2019	25.5	0.00	0
BOM1w0708193	BOM1	7/8/2019	25.5	0.14	1 L
BOM1w0708194	BOM1	7/8/2019	25.5	0.00	0
BOM1w0708195	BOM1	7/8/2019	25.5	0.00	0
BOM1wTB070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.05</b>	<b>0/5</b>
BOM1w1021191	BOM1	10/21/2019	---	0.00	0
BOM1w1021192	BOM1	10/21/2019	---	0.00	0
BOM1w1021193	BOM1	10/21/2019	---	0.00	0
BOM1w1021194	BOM1	10/21/2019	---	0.12	1 L
BOM1w1021195	BOM1	10/21/2019	---	0.12	1 L
BOM1wTB102119	tb	10/21/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.05</b>	<b>0/5</b>
BOM2w0528191	BOM2	5/28/2019	12	0.00	0
BOM2w0528192	BOM2	5/28/2019	12	0.00	0
BOM2w0528193	BOM2	5/28/2019	12	0.00	0
BOM2w0528194	BOM2	5/28/2019	12	0.00	0
BOM2w0528195	BOM2	5/28/2019	12	0.00	0
BOM2wTB052819	tb	5/28/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
BOM2w0708191	BOM2	7/8/2019	24.5	0.00	0
BOM2w0708192	BOM2	7/8/2019	24.5	0.00	0
BOM2w0708193	BOM2	7/8/2019	24.5	0.13	1 L
BOM2w0708194	BOM2	7/8/2019	24.5	0.00	0
BOM2w0708195	BOM2	7/8/2019	24.5	0.00	0
BOM2wTB070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.03</b>	<b>0/5</b>
BOM2w1021191	BOM2	10/21/2019	---	0.00	0
BOM2w1021192	BOM2	10/21/2019	---	0.00	0
BOM2w1021193	BOM2	10/21/2019	---	0.11	1

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake Bomoseen - Asian Clam					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
BOM2w1021194	BOM2	10/21/2019	---	0.00	0
BOM2w1021195	BOM2	10/21/2019	---	0.09	1
BOM2wTB102119	tb	10/21/2019		0.10	1
<b>Average concentration &amp; number of positive samples</b>				<b>0.04</b>	<b>0/5</b>
BOM3w0528191	BOM3	5/28/2019	6	0.13	1
BOM3w0528192	BOM3	5/28/2019	6	0.96	10
BOM3w0528193	BOM3	5/28/2019	6	0.00	0
BOM3w0528194	BOM3	5/28/2019	6	1.27	9
BOM3w0528195	BOM3	5/28/2019	6	0.59	4
BOM3wTB052819	tb	5/28/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.59</b>	<b>3/5</b>
BOM3w0708191	BOM3	7/8/2019	24.5	3.86	27
BOM3w0708192	BOM3	7/8/2019	24.5	6.86	45
BOM3w0708193	BOM3	7/8/2019	24.5	7.24	46
BOM3w0708194	BOM3	7/8/2019	24.5	12.06	114
BOM3w0708195	BOM3	7/8/2019	24.5	2.50	20
BOM3wTB070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>6.50</b>	<b>5/5</b>
BOM3w1021191	BOM3	10/21/2019	---	0.28	3
BOM3w1021192	BOM3	10/21/2019	---	0.00	0
BOM3w1021193	BOM3	10/21/2019	---	0.12	1
BOM3w1021194	BOM3	10/21/2019	---	0.00	0
BOM3w1021195	BOM3	10/21/2019	---	0.00	0
BOM3wTB102119	tb	10/21/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.08</b>	<b>1/5</b>
AC 1:1000				62.62	377
AC 1:10,000				5.14	39
NTC				0.00	0

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake George - Zebra Mussel					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
LG1w0528191	LG1	5/28/2019	12.00	0.00	0
LG1w0528192	LG1	5/28/2019	12.00	0.00	0
LG1w0528193	LG1	5/28/2019	12.00	0.00	0
LG1w0528194	LG1	5/28/2019	12.00	0.00	0
LG1w0528195	LG1	5/28/2019	12.00	0.00	0
LG1tb0528191	tb	5/28/2019	12.00	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG1w0708191	LG1	7/8/2019	21.00	0.00	0
LG1w0708192	LG1	7/8/2019	21.00	0.00	0
LG1w0708193	LG1	7/8/2019	21.00	0.00	0
LG1w0708194	LG1	7/8/2019	21.00	0.00	0
LG1w0708195	LG1	7/8/2019	21.00	0.00	0
LG1tb070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG2w0528191	LG2	5/28/2019	12.00	0.00	0
LG2w0528192	LG2	5/28/2019	12.00	0.00	0
LG2w0528193	LG2	5/28/2019	12.00	0.00	0
LG2w0528194	LG2	5/28/2019	12.00	0.00	0
LG2w0528195	LG2	5/28/2019	12.00	0.00	0
LG2tb0528191	tb	5/28/2019	12.00	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG2w0708191	LG2	7/8/2019	23.00	0.00	0
LG2w0708192	LG2	7/8/2019	23.00	0.00	0
LG2w0708193	LG2	7/8/2019	23.00	0.00	0
LG2w0708194	LG2	7/8/2019	23.00	0.00	0
LG2w0708195	LG2	7/8/2019	23.00	0.00	0
LG2tb070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG3w0528191	LG3	5/28/2019	12.50	0.00	0
LG3w0528192	LG3	5/28/2019	12.50	0.00	0
LG3w0528193	LG3	5/28/2019	12.50	0.00	0
LG3w0528194	LG3	5/28/2019	12.50	0.00	0
LG3w0528195	LG3	5/28/2019	12.50	0.00	0
LG3tb0528191	tb	5/28/2019	12.50	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG3w0708191	LG3	7/8/2019	23.00	0.00	0
LG3w0708192	LG3	7/8/2019	23.00	0.00	0

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake George - Zebra Mussel					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
LG3w0708193	LG3	7/8/2019	23.00	0.00	0
LG3w0708194	LG3	7/8/2019	23.00	0.24	2 L
LG3w0708195	LG3	7/8/2019	23.00	0.32	3
LG3tb070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.14</b>	<b>1/5</b>
LG4w0925191	LG4	9/25/2019	19.00	0.00	0
LG4w0925192	LG4	9/25/2019	19.00	0.00	0
LG4w0925193	LG4	9/25/2019	19.00	0.00	0
LG4w0925194	LG4	9/25/2019	19.00	0.00	0
LG4w0925195	LG4	9/25/2019	19.00	0.00	0
LG4tb092519	tb	9/25/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG5w0925191	LG5	9/25/2019	19.00	0.00	0
LG5w0925192	LG5	9/25/2019	19.00	0.00	0
LG5w0925193	LG5	9/25/2019	19.00	0.00	0
LG5w0925194	LG5	9/25/2019	19.00	0.00	0
LG5w0925195	LG5	9/25/2019	19.00	0.00	0
LG5tb092519	tb	9/25/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
NTC				0.00	0
ZM 1:10,000				1.83	16
ZM 1:1000				30.07	243

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake George - Asian Clam					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
LG1w0528191	LG1	5/28/2019	12.00	0.00	0
LG1w0528192	LG1	5/28/2019	12.00	0.00	0
LG1w0528193	LG1	5/28/2019	12.00	0.00	0
LG1w0528194	LG1	5/28/2019	12.00	0.00	0
LG1w0528195	LG1	5/28/2019	12.00	0.00	0
LG1tb0528191	tb	5/28/2019	12.00	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG1w0708191	LG1	7/8/2019	21.00	0.00	0
LG1w0708192	LG1	7/8/2019	21.00	0.00	0
LG1w0708193	LG1	7/8/2019	21.00	0.00	0
LG1w0708194	LG1	7/8/2019	21.00	0.00	0
LG1w0708195	LG1	7/8/2019	21.00	0.00	0
LG1tb070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG2w0528191	LG2	5/28/2019	12.00	0.00	0
LG2w0528192	LG2	5/28/2019	12.00	0.00	0
LG2w0528193	LG2	5/28/2019	12.00	0.00	0
LG2w0528194	LG2	5/28/2019	12.00	0.00	0
LG2w0528195	LG2	5/28/2019	12.00	0.00	0
LG2tb0528191	tb	5/28/2019	12.00	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG2w0708191	LG2	7/8/2019	23.00	0.00	0
LG2w0708192	LG2	7/8/2019	23.00	0.00	0
LG2w0708193	LG2	7/8/2019	23.00	0.00	0
LG2w0708194	LG2	7/8/2019	23.00	0.00	0
LG2w0708195	LG2	7/8/2019	23.00	0.00	0
LG2tb070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
LG3w0528191	LG3	5/28/2019	12.50	0.50	4
LG3w0528192	LG3	5/28/2019	12.50	0.00	0
LG3w0528193	LG3	5/28/2019	12.50	0.35	3
LG3w0528194	LG3	5/28/2019	12.50	0.23	2 L
LG3w0528195	LG3	5/28/2019	12.50	0.23	2 L
LG3tb0528191	tb	5/28/2019	12.50	0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.26</b>	<b>2/5</b>
LG3w0708191	LG3	7/8/2019	23.00	0.40	3
LG3w0708192	LG3	7/8/2019	23.00	0.88	8
LG3w0708193	LG3	7/8/2019	23.00	0.21	2 L

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake George - Asian Clam					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
LG3w0708193	LG3	7/8/2019	23.00	0.10	1 L
LG3w0708194	LG3	7/8/2019	23.00	0.24	2 L
LG3w0708195	LG3	7/8/2019	23.00	0.64	5
LG3tb070819	tb	7/8/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.41</b>	<b>3/5</b>
LG4w0925191	LG4	9/25/2019	19.00	3.08	30
LG4w0925192	LG4	9/25/2019	19.00	1.79	17
LG4w0925193	LG4	9/25/2019	19.00	1.80	14
LG4w0925194	LG4	9/25/2019	19.00	1.28	12
LG4w0925195	LG4	9/25/2019	19.00	0.76	8
<b>Average concentration &amp; number of positive samples</b>				<b>1.74</b>	<b>5/5</b>
LG5w0925191	LG5	9/25/2019	19.00	1.27	12
LG5w0925192	LG5	9/25/2019	19.00	0.81	8
LG5w0925193	LG5	9/25/2019	19.00	3.00	38
LG5w0925194	LG5	9/25/2019	19.00	2.52	28
LG5w0925195	LG5	9/25/2019	19.00	0.55	7
LG5tb092519	tb	9/25/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>1.63</b>	<b>5/5</b>
AC 1:1000				42.21	596
AC 1:10,000				2.21	31
NTC				0.00	0

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Lake Memphremagog - Zebra Mussel					
Sample	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positive droplets
MG1w082319A	MG1	8/23/2019	17.20	0.00	0
MG1w082319B	MG1	8/23/2019	17.20	0.15	2 L
MG1w082319C	MG1	8/23/2019	17.20	0.00	0
MG1w082319D	MG1	8/23/2019	17.20	0.00	0
MG1w082319E	MG1	8/23/2019	17.20	0.08	1 L
MG1tb082319	tb	8/23/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.05</b>	<b>0/5</b>
MG3w082319A	MG3	8/23/2019	18.9	0.00	0
MG3w082319B	MG3	8/23/2019	18.9	0.00	0
MG3w082319C	MG3	8/23/2019	18.9	0.16	2 L
MG3w082319D	MG3	8/23/2019	18.9	0.00	0
MG3w082319E	MG3	8/23/2019	18.9	0.00	0
MG3tb082319	tb	8/23/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.03</b>	<b>0/5</b>
MG4w082319A	MG4	8/23/2019	18.9	0.00	0
MG4w082319B	MG4	8/23/2019	18.9	0.00	0
MG4w082319C	MG4	8/23/2019	18.9	0.00	0
MG4w082319D	MG4	8/23/2019	18.9	0.00	0
MG4w082319E	MG4	8/23/2019	18.9	0.00	0
MG4tb082319	tb	8/23/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/5</b>
MG5w082319A	MG5	8/23/2019	18.3	0.00	0
MG5w082319B	MG5	8/23/2019	18.3	0.00	0
MG5w082319C	MG5	8/23/2019	18.3	0.00	0
MG5w082319D	MG5	8/23/2019	18.3	0.00	0
MG5w082319E	MG5	8/23/2019	18.3	0.08	1 L
MG5tb082319	tb	8/23/2019		0.00	0
<b>Average concentration &amp; number of positive samples</b>				<b>0.02</b>	<b>0/5</b>
ZM 1:1000				32.94	445
ZM 1:10,000				1.21	17
NTC				0.00	0

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

Great Pond - Asian Clam					
Sample ID	Site	Date Collected	Water Temp °C	Conc(copies/μL)	Positives droplets
GP1082219	Great Pond beach	8/22/2019	18	0.00	0.00
<b>Average concentration &amp; number of positive samples</b>				<b>0.00</b>	<b>0/1</b>
GP20822191	Great Pond ramp	8/22/2019	18	0.71	6.00
GP20822192	Great Pond ramp	8/22/2019	18	0.40	3.00
GP20822193	Great Pond ramp	8/22/2019	18	0.67	7.00
GP20822194	Great Pond ramp	8/22/2019	18	2.33	17.00
<b>Average concentration &amp; number of positive samples</b>				<b>1.03</b>	<b>5/5</b>
AC 1:100				2,997.69	10,321.00
AC 1:1,000				178.23	1,355.00
AC 1:10,000				18.01	152.00
NTC				0.00	0.00

NTC = Negative Control

AC/ZM = Positive control (Asian clam/zebra mussel tissue)

tb = Trip blank

L = Low; fewer than 3 positive droplets

## **APPENDIX B – Environmental DNA (eDNA) Sampling Protocol**

# Environmental DNA (eDNA) Sampling Protocol

## Field filtered samples

### General Field Supplies

- Small cooler with ice – if blue ice is used, wipe off with bleach solution before putting in cooler
- Small trash bag
- Distilled or lab water
- Spray bottle containing 10% bleach
- Small bucket with a 1L line marked inside\*
- Hand or portable electric pump\*
- Waders- if not sampling from a boat\*
- Container to carry equipment\*
- Extra gloves

\*Clean with 10% bleach between sites.

### Per site supplies

- 1 Ziplock baggie with paper towels
- 1 Ziplock baggie filled with disposable gloves (10 pairs/site)
- Pump tubing and connector
- Sample tube (1.5ml microcentrifuge tube with cap); labeled. 1 per sample, and 1 for blank.
- Disposable funnel with 1.5um filter (in a plastic bag-either the one it comes in or a Ziplock)
- Sharpie
- Sampling form
- Garbage bag

### Before Sampling

Label the sample tubes (while wearing gloves) and place in separate ziplock bags for each site  
Check field supplies, put fresh ice in cooler

### Sampling

Designate one person as the Sampler. This person will collect the sample and will have direct contact with the water. A second person will be designated as 'Handler'. This person will assist the Sampler, but will not have direct contact with water (this helps minimize transfer of contamination from the sample to the unused supplies).

1. Fill out field sampling form
2. The person designated as "Sampler" puts on waders that have been cleaned with water and 10% bleach prior to arrival
3. Put on gloves
4. Collect a field blank – Sampler opens one of the sealed filter funnels, exposes it to the site for few minutes, then folds it, and stores in a capped tube as described below

5. Collect sample - Handler sets up the pump, Sampler attaches the disposable funnel, then wades into the stream to collect a sample. Sample funnel should be held about 10cm below the surface of the water, and moved gently around to maximize the region sampled. Handler operates the pump and holds the bucket.
6. When 1L has been filtered through, the Sampler removes the top of the funnel (can go into the trash bag), then removes and folds the filter (with his/her gloved hands and forceps) into half, then half again, then half again and one last time half again for a total of four folds.
7. The Handler puts on clean gloves, gets the sample tube from the kit, and holds it while the Sampler carefully places the filter into it.
8. The Handler closes the sample tube and places it in a ziplock bag in the cooler (on ice)
9. Disassemble and wipe down the pump, hose, and bucket with bleach solution and paper towels
10. The Sampler rinses the waders in the water, removing mud and other big debris and removes them. Waders should be sprayed with 10% bleach and wiped with papers towels.
11. Continue on to the next sampling site being careful to keep the waders, pump and bucket away from the rest of the supplies to avoid contamination from site to site

**At the next site:**

- The person designated as “Sampler” puts on waders that have been cleaned with water and 10% bleach prior to arrival
- From the shore, the Handler fills the bucket with water and then rinses the waders that the Sampler is wearing – make sure the water doesn’t run off into your sample site
- From here continue following the instructions as above, making sure to clean the waders, pump, bucket, and anything else that has contacted the water thoroughly between each sampling site

**After sampling:**

- Freeze filters as soon as possible
- Wash and decontaminate field equipment using tap water and bleach spray. Store sampling supplies in a clean area separate from possible contamination.
- If samples are being shipped for analysis, pack them in dry ice or blue ice to avoid meltwater during shipping. Samples should be sent overnight, and carefully tracked to ensure they arrive as soon as possible.

**Notes:**

Bleach destroys DNA, so be careful not to let it contact your sample or filter!

### Sample Collection

Location: \_\_\_\_\_

Date: \_\_\_\_\_

Sampling Team: \_\_\_\_\_

Weather/comments: \_\_\_\_\_

-----  
Site ID: \_\_\_\_\_

Other ID: \_\_\_\_\_

Water Temp: \_\_\_\_\_

Sample Depth: \_\_\_\_\_

#### Samples collected:

Sample ID:	Volume:	Comments

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

## **APPENDIX C – Analytical Methods and Species Specific Assays**

## Appendix C - Analytical Methods and Species Specific Assays

Analyses were performed on a Biorad Digital Droplet PCR system (ddPCR) using species specific assays. Initially, zebra mussel analyses were conducted with an assay described in Amberg et al. (2019), but after review of results and further discussion with personnel at the USGS a *Dreissena* ssp. assay (DRE16S, Gingera et al. 2017) was adopted. This analysis does not distinguish between zebra mussels and quagga mussels, but may have lower detection limits than other published assays (Supulveda et al. 2019).

The *C. fluminea* primers are based on Cowert et al. 2018, modified to align with a higher number of sequences in the NCBI database. We aligned the published primers with the available *C. fluminea* reference sequences from the NCBI nucleotide database and found some sequences with mismatches to both primers. The forward and reverse primers were modified by adding degenerate nucleotides to correct these mismatches. The forward was changed from TTTATTAGATGATGGGCAGCTGTA to ttaytrgatgatgggcagytgta; an example of a sequence that this would match that was not matched by the original primer is DQ264393. The reverse was changed from TGATCTAACCAACAAAAGCATAGC to tgatctmacyaacaaaagcatagc. Sequences with mismatches to the original primer include KT893363 and KT893352. Two ddPCR probes were designed corresponding to two sequence variants in the *C. fluminea* reference sequences, agggctcctgatatagctttccac and agggctcctgatatggctttcctc.

### *Dreissena* ssp. assay (DRE16S, Gingera et al. 2017)

Forward: TGGGGCAGTAAGAAGAAAAAATA A  
Reverse: CATCGAGGTCGCAAACCG  
Probe: CCGTAGGGA AACAGC

### *C. fluminea* assay modified from Cowert et al 2018

Forward: TTAYTRGATGATGGGCAGYTGTA  
Reverse : TGATCTMACYAACAAAAGCATAGC  
probe1: AGGGCTCCTGATATAGCTTTCCAC  
probe2: AGGGCTCCTGATATGGCTTTCCCTC

ddPCR Droplet digital PCR was prepared according to the ddPCR Supermix for Probes protocol (Bio-Rad) using two microliters of undiluted sample extract sample. Each set of reactions included a no template control (NTC) with reagent-grade water and positive controls of diluted tissue from the target organism. Reactions were scaled up to 24  $\mu$ L for preparation and 20  $\mu$ L was used for droplet generation in the QX200 Droplet Generator (Bio-Rad). The plate was sealed with the PX1 PCR Plate Sealer (Bio-Rad) and the reaction was performed in a C1000 Touch Thermal Cycler (Bio-Rad) following the cycling profile outlined in the ddPCR Supermix for Probes protocol (Table 1).

**Table 1. ddPCR Cycling Profile** The lid was heated to 105°C and the ramp rate was 2°C/sec for every step.

Step	Temperature (°C)	Time	Number of Cycles
Enzyme activation	95	10 min	1
Denaturation	94	30 sec	40
Annealing/Extension	60	1 min	40
Enzyme Deactivation	98	10 min	1
Hold	4	∞	1

## **APPENDIX D – Field data sheets**

### eDNA Sample Collection

Location: Lake Bomoseen 01 Date: 7/8/19  
Float bridge rd. south west side of bridge  
Sampling Team: Ryan C. & Meg M.

Weather/comments: Clear, sunny 68°F light wind

Site ID: BM 01 Other ID: \_\_\_\_\_

Water Temp: 78°F Depth: 10cm in 2 1/2 ft in waders

#### Samples collected:

Sample ID:	Volume:	Comments
BM 01A	1 lt.	
BM 01B	1 lt.	got rip in hose from pump house.
BM 01C	1 lt.	
BM 01D	1 lt.	
BM 01E	1 lt.	
BM 01 BLANK	air	

Notes: hose some times gets pulled through  
ripped open but workable  
heavy plant growth, found open spot to sample

eDNA Sample Collection

Location: Lake Bomoseen 2 Date: 7/8/19

Sampling Team: Ryan C. + Meg M.

Weather/comments: 76°F Sunny clear

Site ID: BM 02 (VTFWD access) Other ID: \_\_\_\_\_

Water Temp: 76°F Depth: 1ft.

Samples collected:

Sample ID:	Volume:	Comments
BM02A	1lt.	
BM 02B	1lt.	
BM 02C	1lt.	
BM 02D	1lt.	
BM 02E	1lt.	
BM 02 blank	air	

Notes: clear water filter worked much faster

eDNA Sample Collection

Location: Lake Bomoseen 03 Date: 7/8/19  
Docks at Kehoe center

Sampling Team: Ryan C. + Meg M.

Weather/comments: clear, sunny, 76°F

Site ID: Kehoe camping dock Other ID: \_\_\_\_\_

Water Temp: 76°F Depth: off dock, 10cm

Samples collected:

Sample ID:	Volume:	Comments
BM 03 A	1 lt.	
BM 03 B	1 lt.	
BM 03 C	1 lt.	
BM 03 D	1 lt.	
BM 03 E	1 lt.	
BM 03 blank	air	

Notes: efficient sampling off docks

### eDNA Sample Collection

Location: BOMUSEEN

Date: 5/28/19

Sampling Team: Anne Davis, Meg Medley, Kim Jensen, DK, Amy, Edele

Weather/comments: Light Rain - overcast

Site ID: RUM-01-~~A~~BCDE

Other ID: \_\_\_\_\_

Water Temp: 11.0°

Depth: 10" - wading

#### Samples collected:

Sample ID:	Volume:	Comments
B0-01-TB	-0	Curly w/ al flora
B0-01-A	1/16	
B0-01-B	1/16	
B0-01-C	1/16	
B0-01-D	1/16	
B0-01-E	1/16	

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

### eDNA Sample Collection

Location: Bumasean

Date: 5/28/19

Sampling Team: DB, AB, AW, Perry, KJ, Spencer

Weather/comments: Rain

Site ID: BOM-02TRARIDE

Other ID: \_\_\_\_\_

Water Temp: 12.0°

Depth: 0"

#### Samples collected:

Sample ID:	Volume:	Comments
BOM-02-TR	—	
BOM-02-A	1L	Rocky Shallow
BOM-02-B		
BOM-02-C		
BOM-03-D		
BOM-03-E		

Notes: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

### eDNA Sample Collection

Location: BUMOSEAN

Date: 5/29/19

Sampling Team: DB, MM

Weather/comments: Rain / calm

Site ID: BUM-03 TRABCOE

Other ID: \_\_\_\_\_

Water Temp: 6°

Depth: DOCK - 2'

#### Samples collected:

Sample ID:	Volume:	Comments
BUM-03 TR		
BUM-03-A		
" " B		
" " C		
" " D		
" " E		

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

### eDNA Sample Collection

Location: Lake Bomoseen

Date: 10/21/19

Sampling Team: Ryan Colarusso

Weather/comments: \_\_\_\_\_

.....  
Site ID: BOM-1

Other ID: \_\_\_\_\_

Water Temp: NA

Depth: \_\_\_\_\_

#### Samples collected:

Sample ID:	Volume:	Comments
BOM1-1	1l	
BOM1-2	1l	
BOM1-3	1l	
BOM1-4	1l	
BOM1-5	1l	
BOM1-6	-	

Notes: Form filled out by A. Watts, based on email information

eDNA Sample Collection

Location: Lake Bomoseen

Date: 10/21/19

Sampling Team: Ryan Colarusso

Weather/comments: \_\_\_\_\_

.....  
Site ID: Bom2

Other ID: \_\_\_\_\_

Water Temp: \_\_\_\_\_

Depth: \_\_\_\_\_

Samples collected:

Sample ID:	Volume: (L)	Comments
Bom2-1	1	
Bom2-2	1	
Bom2-3	1	
Bom2-4	1	
Bom2-5	1	
Bom2-6	—	

Notes: Form filled out by A. Watts

\_\_\_\_\_  
\_\_\_\_\_

eDNA Sample Collection

Location: Lake Bomoseen Date: 10/21/19

Sampling Team: Ryan Colarusso

Weather/comments: \_\_\_\_\_

.....  
Site ID: BOM3

Other ID: \_\_\_\_\_

Water Temp: \_\_\_\_\_

Depth: \_\_\_\_\_

Samples collected:

Sample ID:	Volume:	Comments
BOM3-1	1	
BOM3-2	1	
BOM3-3	1	
BOM3-4	1	
BOM3-5	1	
BOM3-6	—	

Notes: Form filled out by A. Watts, based on  
emailed information

Sample Collection

-73.08545646

43.90709976

Location: Lake Dunmore, Vermont Date: 10/16/19  
Salisbury, VT F&W Magoon Access Area

Sampling Team: Kim Tensen, Ryan Colarusso, Lisa Cichetti

Weather/comments: Sunny at beginning of sampling, turning cold & cloudy  
62°F 65°F

Site ID: \_\_\_\_\_

Other ID: \_\_\_\_\_

Water Temp: 51°F

Sample Depth: ~ 10 cm below water

Samples collected:

Sample ID:	Volume:	Comments
1		Blank
2	3L	Sample taken on North side of Dock
3	3L	Sample taken on East side of Dock
4	3L	Sample taken on West side of Dock, where ZM were harvested
5	3L	"

Notes: Samples were taken around VT Fish & Wildlife Access Area dock where a new infestation/occurrence of ZM were found, identified, and harvested in September, 2019. Approximately 200 specimens harvested.



eDNA Sample Collection

Location: Great Pond, NH

Date: 8/22/19

Sampling Team: Alison Watts, Heather Gilbert, UNH

Weather/comments: Sunny

Site ID: GPI

Other ID: \_\_\_\_\_

Water Temp: 18C

Depth: ~ 1ft

Samples collected:

Sample ID:	Volume:	Comments
GPI0822191	21	
<del>GPI0822192</del>		
<del>GPI</del>		

Notes: sample from beach area

\_\_\_\_\_  
\_\_\_\_\_

**eDNA Sample Collection**

**Location:** Great Pond, NH      **Date:** 8/22/19

**Sampling Team:** Alison Watts, Heather Gilbert, UNH

**Weather/comments:** sunny

**Site ID:** GPR

**Other ID:** \_\_\_\_\_

**Water Temp:** 18°C

**Depth:** \_\_\_\_\_

**Samples collected:**

Sample ID:	Volume:	Comments
GP20822191	2 l	} north side of boat ramp
GP20822192	2 l	
GP20822193	2 l	} south side of boat ramp
GP20822194	2 l	

**Notes:** clam shells visible

eDNA Sample Collection

Location: Huletts Landing Date: 5/28/19

Sampling Team: Meg/Modley/Steven Pearson

Weather/comments: Cold 50° pouring rain

Site ID: LG 01

Other ID: \_\_\_\_\_

Water Temp: 12°C

Depth: 4 ft

Samples collected:

Sample ID:	Volume:	Comments
LG 01 D	1 L	filter became detached inside filter cup at end of sampling event

Notes: Sample taken from Huletts Landing Marina  
(43.644472, -73.506644) off dock just north  
of swim area

6065 Lakeside Way, Huletts Landing, NY 12841

eDNA Sample Collection

Location: Pilot Knob Beach Date: 5/28/19 4:30pm

Sampling Team: Meg Modley / Steven Pearson

Weather/comments: 50°F raining

Site ID: LG 02

Other ID: \_\_\_\_\_

Water Temp: 12°C

Depth: 1 ft.

Samples collected:

Sample ID:	Volume:	Comments

Notes: Sample site: 1919 Co Rd. 32 Fort Ann, NY  
(43.519272, -73.626894) 12827

eDNA Sample Collection

Location: Million Dollar Beach Date: 5/28/19 5:30pm

Sampling Team: Meg Modley / Steven Pearson

Weather/comments: 50°F raining

.....  
Site ID: LG 03

Other ID: \_\_\_\_\_

Water Temp: 12.5°C

Depth: 1 ft.

Samples collected:

Sample ID:	Volume:	Comments
LG 03 C	Slightly < 1 ft.	the pump was very slow more turbid water
LG 03 E	Slightly < 1 ft.	Killed battery on drill / pump just enough power to get 1 ft. unable to pump remaining water through filter to "dry" it.

Notes: Samples taken from west edge of Million Dollar Beach (43.418452, -73.705586)

Beach Rd, Lake George, NY 12845

eDNA Sample Collection

Location: Lake George - Hubbs Landing Date: 7/8/19

Sampling Team: Meg Modley, Steven Pearson

Weather/comments: Sunny / 78° F

Site ID: 01

Other ID: \_\_\_\_\_

Water Temp: 70° F

Depth: 3.5 ft.

Samples collected:

Sample ID:	Volume:	Comments
LGØ1A	1 lt.	
LGØ1B	1 lt.	
LGØ1C	1 lt.	
LGØ1D	1 lt.	
LGØ1E	1 lt.	
LGØ1 Blank	air	

Notes: end of the dock next to swim area

### eDNA Sample Collection

Location: Lake George O2-Plots Knob Date: 7/8/19

Sampling Team: Meg Modley, Steven Pearson

Weather/comments: Sunny 82°F

Site ID: LG02

Other ID: \_\_\_\_\_

Water Temp: 74°F

Depth: 1ft.

#### Samples collected:

Sample ID:	Volume:	Comments
LG02A	1 L.	
LG02B	1 L.	
LG02C	1 L.	tube was eaten by vacuum pump
LG02D	1 L.	
LG02E	1 L.	
LG02 blank	air	

Notes: more sediment in water here

lots of swimmers at beach

eDNA Sample Collection

Location: Lake George 03 Date: 7/8/19

Million dollar beach - launch site east  
Sampling Team: Meg M. + Steven P.

Weather/comments: 84°F hot, sunny, light wind

Site ID: LG 03

Other ID: —

Water Temp: 74°F

Depth: 1ft.

Samples collected:

Sample ID:	Volume:	Comments
LG03A	1 lt.	
LG03B	1 lt.	
LG03C	1 lt.	
LG03D	1 lt.	
LG03E	1 lt.	
LG03blank	air	

Notes: sampling in shade

### eDNA Sample Collection

Location: Beckleys Marina Date: 9/24/19  
Boat Ramp

Sampling Team: Meg Modley, Matt Cosby

Weather/comments: 65°F - calm, partly cloudy

Site ID: LG 5 Other ID: \_\_\_\_\_

Water Temp: 19°C

Depth: 4-6 inches depth - from end of dock at boat launch water depth 3 feet

**Samples collected:**

Sample ID:	Volume:	Comments
LG 5 A	1 liter	
LG 5 B	1 liter	
LG 5 C	1 liter	tubing pulled through device and punctured
LG 5 D	1 liter	
LG 5 E	1 liter	
LG 5 BLK	exposed to air	

Notes: Asian clam shells visible, no zebra mussels observed.

### eDNA Sample Collection

Location: Rogers Rock Campground Date: 9/24/19

Sampling Team: Meg Modley, Matt Cosby

Weather/comments: 68°F - overcast, partially sunny, mild wind  
time - 3:30 pm

Site ID: LG 4

Other ID: \_\_\_\_\_

Water Temp: 19°C

Depth: 6 inches depth off boat  
launch dock at 3-4ft. depth

#### Samples collected:

Sample ID:	Volume:	Comments
LG 4 A	1 liter	
LG 4 B	1 liter	
LG 4 C	1 liter	
LG 4 D	1 liter	
LG 4 E	1 liter	
LG 4 BLK	fell in water	- no pumping, blank fell into water

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

### Sample Collection

Location: Memphremagog

Date: 8/23/19

Sampling Team: RTC + LC

Weather/comments: Sunny, partly cloudy, breezy

Site ID: MG01

Other ID: Pointe Merry

Water Temp: 63°F

Sample Depth: ~10cm

#### Samples collected:

Sample ID:	Volume:	Comments
MG01 blank	—	
MG01 A	1L	
MG01 B	1L	
MG01 C	1L	
MG01 D	1L	
MG01 E	1L	

Notes: Very shallow site, lots of silt in samples

Sample Collection

Location: Memphremagog

Date: 8/23/19

Sampling Team: ZTC + LC

Weather/comments: Sunny, slight breeze

.....  
Site ID: M602

Other ID: Magog Municipal Water Pump

Water Temp: \_\_\_\_\_

Sample Depth: \_\_\_\_\_

Samples collected:

Sample ID:	Volume:	Comments
M602 blank		
M602 A		
M602 B		
M602 C		
M602 D		
M602 E		

Notes: COULD NOT ACCESS

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Sample Collection

Location: Memphremagog

Date: 8/23/19

Sampling Team: BTC + LC

Weather/comments: Sunny, slight breeze

Site ID: MG03

Other ID: Georgetown

Water Temp: 66°F

Sample Depth: ~10cm

Samples collected:

Sample ID:	Volume:	Comments
MG03 blank	—	
MG03 A	1L	
MG03 B	1L	
MG03 C	1L	
MG03 D	1L	
MG03 E	1L	

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

### Sample Collection

Location: Memphremagog

Date: 8/23/19

Sampling Team: BTC+LC

Weather/comments: Partly cloudy, sunny, light breeze

Site ID: MG04

Other ID: Cedarville

Water Temp: 66°F

Sample Depth: \_\_\_\_\_

#### Samples collected:

Sample ID:	Volume:	Comments
MG04 blank	-	
MG04 A	1L	
MG04 B	1L	
MG04 C	1L	
MG04 D	1L	
MG04 E	1L	

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

### Sample Collection

Location: Memphrenagog

Date: 8/23/19

Sampling Team: RTC + LC

Weather/comments: Mostly cloudy, light breeze

Site ID: MG05

Other ID: Newport City Dock

Water Temp: 65°F

Sample Depth: \_\_\_\_\_

#### Samples collected:

Sample ID:	Volume:	Comments
MG05 blank	—	
MG05 A	1L	
MG05 B	1L	
MG05 C	1L	
MG05 D	1L	
MG05 E	1L	

Notes: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_