RECLAMATION

Managing Water in the West

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PCR Detection of Quagga Mussel Intracellular DNA and Dissolved DNA

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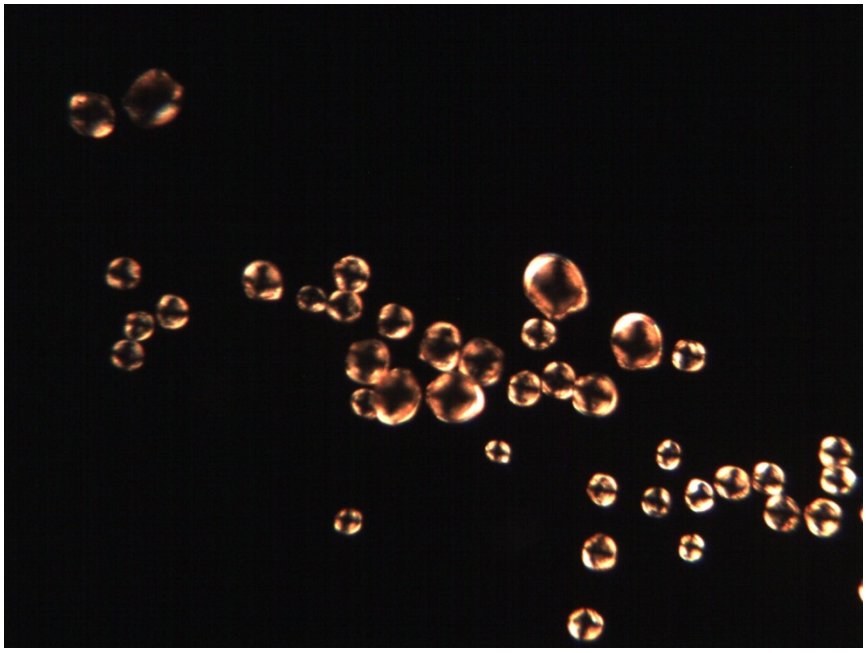

Quagga mussel veligers under cross polarized light microscopy

Jamie Carmon, Jacque Keele, Sherri F. Pucherelli, Denise Hosler

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Mission Statements

The U.S. Department of the Interior protects America's natural resources and heritage, honors our cultures and tribal communities, and supplies the energy to power our future.

The mission of the Bureau of Reclamation is to manage, develop, and protect water and related resources in an environmentally and economically sound manner in the interest of the American public.

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Executive Summary

The Reclamation Detection Laboratory for Exotic Species (RDLES) utilizes both microscopy and PCR to detect presence of dreissenid mussels in western water bodies. PCR results are variable and there are times where it is possible to get a negative result by microscopy and a positive by PCR on the same sample. The goal of this study was to demonstrate how multiple factors (extraction kit type, amount of DNA in the sample, days to analysis, and presence or absence inhibitors) impact the success rate of detecting quagga mussel environmental DNA (eDNA). PCR success rate was observed in four different scenarios: detection of whole veliger bodies, broken veligers, degraded veligers, and two concentrations of free floating adult DNA in water with and without inhibitors added. Overall, the results of this study indicate that it is possible to achieve a positive PCR result on a water sample that was found to be negative by microscopy, because PCR can detect free floating, dissolved DNA (dDNA) and veligers that are degraded and broken apart. While microscopy is an import aspect of dreissenid early detection, this study indicates that PCR testing of dreissenid early detection samples is a valuable tool that is capable of detecting signs of dreissenid presence that would otherwise be missed by microscopy alone.

Contents

Introduction

The quagga mussel (*Dreissena rostriformis bugensis)* is an introduced freshwater bivalve that is spreading across the western United States, affecting water ecology and impacting infrastructure such as dams, water intakes, and water treatment facilities. The adult mussels are between 6 and 45 millimeters in length and prefer to live below the photic zone, making detection of adult mussels difficult (Army Corps of Engineers, 2002; Claudi & Mackie, 1998). The accepted method for quagga mussel early detection is to sample for the larval (veliger) life stage (80-600 microns) (Army Corps of Engineers, 2002). Raw water samples are traditionally collected with a 64-µm plankton tow net because veligers are free floating in the water column until they develop into juveniles and settle on substrates (Claudi & Mackie, 1998; Army Corps of Engineers, 2002).

The Reclamation Detection Laboratory for Exotic Species (RDLES) is dedicated to the early detection of invasive, threatened, and endangered species across the United States. RDLES has been one of the leaders in advancing the science of early detection of invasive mussels. Cross polarized light microscopy (CPLM) is the preferred and standard way of detecting veligers in raw water samples (Army Corps of Engineers, 2002; Claudi & Mackie, 1998; Carmon & Hosler, B, 2013). However, degradation of the veliger shell may result in false negative microscopy findings (Carmon, Keele, Pucherelli, & Hosler, 2014). Veligers are microscopic, which makes identification difficult, especially if other bivalves or similar organisms are present in the water sample. For example, it is difficult to differentiate between zebra and quagga mussels veligers by microscopy. Due to this uncertainty, RDLES has included polymerase chain reaction (PCR) testing for the detection of DNA in addition to the microscopy analysis of raw water samples. This test helps reduce the likelihood of false negatives and verifies species identification, as needed. PCR is also capable of detecting the presence of DNA, even from veligers that are degraded beyond the point of microscopic detection.

When collecting DNA samples from general sources such as water, soil, rock, or air, the type of sampling is called environmental DNA (eDNA) sampling (Bellemain, 2013; Thomsen, 2013). In order to identify an organism by microscopy, most, or all, of the organism’s body must be present in the sample, but PCR only requires a few copies the DNA to be present. Detection of target DNA can result from the presence of the organism’s whole body, a piece of the body, a few sloughed cells, or free floating DNA in the sample (Bellemain, 2013; Herder, 2013). eDNA that is encapsulated within a cell is referred to as intracellular DNA (iDNA), and free floating DNA is called dissolved DNA (dDNA) (Matsui, Honjo, & Kawabata, 2001). Currently there is no way to distinguish whether a positive PCR result is from iDNA, dDNA, or even ancient DNA that has been in the water for many years.

When sampling for eDNA in a water source, surface water samples are typically collected in order to exclude ancient DNA, or iDNA. Surface dDNA will degrade at around 170 hours, due to UV light and endonucleases, while dDNA located at the bottom of a water body seems to degrade much slower (Matsui, Honjo, & Kawabata, 2001). Dissolved DNA in the hypolimnion can be caught up with other DNA, tissue, and sediments. Uptake of dDNA in bacteria is also common (Matsui, Honjo, & Kawabata, 2001; Nielsen, Johnsen, Bensasson, & Daffonchio, 2007; Dejean, et al., 2011). While RDLES is interested in collecting dreissenid eDNA, the main objective of quagga mussel early detection sampling is to collect the veliger body so that it can be observed by microscopy. Therefore, RDLES samples the entire water column in order to collect both the veliger body and any free floating DNA.

When early detection samples arrive at RDLES they are first analyzed by microscopy then by PCR. The first step of PCR analysis is DNA extraction, and there are many commercial DNA extraction kits that are specifically optimized for different types of collection media. At RDLES, the DNeasy blood and tissue kit (Qiagen, 2013-2014) is used to isolate DNA from individual suspects (veligers) found by microscopy. RDLES uses the power soil kit (MoBio Laboratories, INC, 2013) to extract DNA from raw water samples containing a variety of non-target DNA sources and inhibitors.

Quagga and zebra mussel species specific primers are used to amplify a region of the COI gene. This gene is popular for species identification as it is a highly conserved mitochondrial gene, and cells have multiple copies. After amplification, the polymerase chain reaction (PCR) product is run on agarose gel and a band of a known size will occur on the gel if the target DNA was amplified. The PCR product can also be sent for gene sequencing, which confirms the species identification (Keele, Carmon, & Hosler, 2013).

Detection of low numbers of veligers by PCR is difficult to consistently achieve, and is likely due to the small amount of tissue in a veliger and the presence of PCR inhibitors (such as zooplankton and humic acids) in the raw water. However, diluting inhibitors can over dilute the DNA resulting in false negatives, while under diluting the inhibitors affects the chemistry of the PCR (Beja-Pereira, Oliveira, Alves, Schwartz, & Luikart, 2009).

The goal of this study was to demonstrate how multiple factors (extraction kit type, amount of DNA in the sample, days to analysis, and presence or absence inhibitors) impact PCR detection of quagga mussel iDNA and dDNA. PCR success rate was observed in four sample scenarios including detection of whole veliger bodies in water without inhibitors and in water with inhibitors, and detection of broken veligers, degraded veligers, and two concentrations of free floating adult DNA in water with inhibitors. These tests will help to explain the variability of quagga mussel early detection PCR test results.

Methods

Raw Water, Zooplankton, and Veliger Collection and Preparation

The raw water and zooplankton used for this study were collected at Chatfield Reservoir, Denver, CO. Surface water was filtered through a 65-µm sieve to filter out debris and zooplankton. Zooplankton were collected from plankton tow samples (64-µm) and were euthanized by freezing.

The quagga mussel veligers used for this study were collected at Lake Mead, Boulder City, Nevada using a 64-µm plankton tow net. The veligers were preserved with 20% isopropanol alcohol, per water volume, and buffered with 0.2 grams of baking soda per 100 mL of liquid (Carmon & Hosler, A, 2013). Samples were shipped overnight, on ice to RDLES, where the veligers were stored at 4°C. Only veligers that were birefringent (visible under CPLM), intact, and containing tissue were used to create the studies' veliger stock.

To create degraded veligers, a subsample of approximantly1000 veligers was taken from the veliger stock and exposed to an acidic solution in order to degrade the shell so that it was no longer visible under CPLM. To produce this effect, veligers were exposed to a solution consisting of 500 µL of deionized (DI) water and 300 µL of 5% acetic acid for 10 minutes. Diluting the acetic acid wash allowed for slower loss of shell degradation. Once the majority of veligers became negative by cross polarized light microscopy, all veligers were washed three times in a fresh DI water bath, and were stored in DI water with 20% alcohol and buffer.

A second subset of veligers was broken apart so that they were undetectable by microscopy. This process was completed in the sample tube in which the DNA extraction would take place so that DNA would not be lost during transfer. The appropriate numbers of veligers were added to pre-labeled DNA extraction tubes containing 30 inert glass beads (USA Scientific 7400-2405). Tubes were placed and shaken on the Vortex Genie (Mo Bio Laboratory) at maximum speed for 15 minutes. The content of every tenth sample was examined by microscopy to ensure that the veligers were destroyed beyond recognition.

Blood and Tissue Kit and Soil Kit Analysis of iDNA

The blood and tissue kit (Qiagen, 2013-2014) and soil kit (MoBio Laboratories, INC, 2013) are both used to extract DNA from a sample. The kits' effectiveness were compared by testing DNA detection of whole veligers (veligers that were intact and birefringent), degraded veligers (not birefringent/ degraded shell), and broken veligers (body not detectable by microscopy) in raw water containing zooplankton. In parallel, whole veligers, in DI water, were tested as a control, because DI water does not contain DNA inhibators like raw water. Each of these

scenarios were tested with both 1 veliger and 25 veligers. For each veliger scenerio, DNA detection was examined in replicates of three, along with a fourth blank sample (a control replicate that did not contain any veligers). Each replicate was tested with each DNA extraciton kit, on day 2, 21, and 42 after veligers were placed into the test conditions. These timepoints were selected in order to compare DNA detection of freshly collected veligers (2 days), veligers at the average hold time at RDLES (21 days), and veligers at more long-term holding times (42 days).

Blood and Tissue Kit Extraction

Two-mL tubes (FisherBrand 05-408-146) were labeled with the number of veligers (1 or 25), the date for sample analysis (Day 2, 21 and 42), and the veliger scenario (whole veliger in DI water, whole veliger in raw water, degraded veliger in raw water, or broken veliger in raw water). The appropriate amount and type of veligers were added to each tube along with 250 µL of raw water solution (raw water with 20% isopropanol alcohol, and buffer to replicate preserved samples) and 0.4 grams of zooplankton (or in the case of the DI water scenario, 250 µL of DI water solution composed of DI water 20% isopropanol alcohol and buffer, and no zooplankton).

On the day of anlysis, the lysis buffer was added to the tube to begin lysis. Samples analyzed with the blood and tissue kit were processed according to the FDA SOP for DNA Extraction and Fish Barcoding (U.S. Food and Drug Administration, 2011). The ATL and Proteinase K were added directly to the tube containing the sample, which was then incubated for 3 hours at 37^{o}C. Following the extraction, the DNA was stored at 4^{o}C until it was analyzed by PCR.

Soil Kit Extraction

Two-mL screw top bead beating tubes (Mo Bio 12800-200-E), empty of lysis buffer and beads, were labeled and prepared in the same way as was done for the blood and tissue kit. On the day of analysis, the beads and extraction fluid from the soil kit were placed in the tube to begin lysis. DNA was extracted using the soil kit and according to manufacturer's instructions. Following the extraction, DNA was stored at 4^{o}C until it was analyzed by PCR.

Blood and TissueKit and Soil Kit Analysis of dDNA

The DNA used for this study was collected from three adults collected at Lake Mead and Lake Mohave, NV. The DNA was extracted from the adductor muscle using the Food and Drug Administration's (FDA) standard operating procedure (SOP) for DNA Extraction for Fish Barcoding (U.S. Food and Drug Administration, 2011). After extraction, the DNA absorbance (260 nm) was taken with a Beckman DU 650 Spectrophotometer to estimate the DNA concentration in each extract. The wavelength of 260 nm was used with the Beer Lambert Law to estimate the concentration of the double stranded DNA in the extract. The DNA concentration in each extract was low, so the replicates were combined to make DNA stocks of 5 ng/µl and 80 ng/µl. The DNA stocks were tested in

triplicate to determine a baseline PCR response, as per the RDLES PCR SOP (Keele, Carmon, & Hosler, 2013). After confirming the DNA stocks produced positive PCR results, three replicates (and a blank control without DNA) of each DNA concentration were prepared for DNA extraction by each kit. Samples were prepared by adding 100 µL of each stock into 2-mL sample tubes with 250 µL of raw water solution (raw water with 20% isopropanol alcohol, and buffer to replicate preserved samples) and 0.4 grams of zooplankton. The DNA was analyzed with the blood and tissue kit and the soil kit as described above.

PCR Analysis

The extracted DNA samples were analyzed for the presence of the quagga mussel COI gene (Keele, Carmon, & Hosler, 2013). The primers for quagga COI are F334 5'-GAAACTGGTTGGTCCCGATA-3' and R335 5'-TAAGGCACCGGCTAAAACAG-3'. PCR was performed using the following master mix: 9.8 µL molecular grade water (Fisher Scientific, 2010), 2 µL of 10X buffer (Fisher Scientific, 2009), 1.6 µL dNTPs (2.5 µM) (Promega, 2014), 1 µL each of the forward and reverse primers (10 µM) (Integrated DNA Technologies (IDT) PCR Primers, 2014), 2.4 µl $MgCl_2$ (25 mM) (New England BioLabs, 2010), and 0.2 µL of Ampli Gold (Roche Diagnostics, 2006) and 2 µL of template DNA. The following PCR program was used: pre-heat 95°C for 9 min, followed by 40 cycles of [95°C for 20 sec, 59°C for 90 sec, and 72°C for 90 sec], then 72°C for 10 min, and finally held at 4°C indefinitely (Keele, Carmon, & Hosler, 2013). Each DNA extract was analyzed three times with independent master mixes for each replicate. If the control failed, the three replicates were repeated, totaling six replicates for some samples.

Results

Blood and Tissue Kit and Soil Kit Analysis of iDNA

Whole Veligers

The whole veliger tests indicated that the blood and tissue kit was more successful at extracting veliger DNA. The samples containing 25 veligers had 100% PCR success rate at 2, 21, and 42 days after collection. In comparison, samples containing 1 veliger had reduced success after day 2. Samples containing 25 veligers had enough DNA present where the type of water (DI vs. raw) did not impact detection. Raw water appeared to reduce the PCR success rate when there is only one veliger present in the sample, as PCR success rate was greater for whole veligers in DI water compared to raw water. But in both cases PCR detection decreased as the time from collection to analysis increased (Table 1, Figure 1).

In general, the soil kit was most successful at detecting whole veligers in DI water, as DNA was detected at 2, 21, and 42 days after collection for the 1 and 25 veliger samples (Table 1, Figure 1). The soil kit did detect veligers in raw water. Samples containing one veliger had an 89% PCR success rate on day 2, but by

day 21 and 42, 0% of samples tested positive. Raw water samples containing 25 veligers had a 100% success rate on day 2, 11% on day 21, and 0% by day 42.

Broken Veligers

When the DNA of raw water samples containing 25 veligers was extracted with the blood and tissue kit, all of the samples produced positive PCR results. Samples containing 1 veliger had lesser PCR success and the results were more variable, as 50% of samples were positive on day 2 and 21, and 89% were positive on day 42. The soil kit also produced variable results over time and between sample sizes, suggesting that the number of veligers and the number of days post collection are not the only source of variability in the test (Table 1, Figure 1).

Degraded Veligers

Raw water samples containing 1 and 25 degraded veligers produced 100% positive PCR results with the blood and tissue kit on day 2, but samples containing 25 veligers decreased to 39% and 33% on days 21 and 42 respectively. Samples containing 1 veliger saw variable success rates as detection decreased to 11% on day 21 and then increased to 78% positive on day 42. The soil kit produced relatively high detection rates on day 2 for samples with 1 and 25 veligers but detection fell to 0% on day 21, but samples containing 25 veligers saw 22% PCR positives again on day 42 (Table 1, Figure 1).

Table 1: Percent of PCR positive results in samples containing 1 or 25 whole, broken, and degraded veligers extracted with the blood and tissue kit and soil kit.

Whole Veligers	**Blood and Tissue Kit**	**Soil Kit**
1 Whole Veliger (DI Water)		
Day 2	100%	94%
Day 21	72%	44%
Day 42	39%	100%
1 Whole Veliger (Raw Water)		
Day 2	100%	89%
Day 21	6%	0%
Day 42	0%	0%
25 Whole Veligers (DI Water)		
Day 2	100%	72%
Day 21	100%	78%
Day 42	100%	44%
25 Whole Veligers (Raw Water)		
Day 2	100%	100%
Day 21	100%	11%
Day 42	100%	0%
Broken Veligers	**Blood and Tissue Kit**	**Soil Kit**
1 Broken Veliger		
Day 2	50%	67%
Day 21	50%	67%
Day 42	89%	78%
25 Broken Veligers		
Day 2	100%	100%
Day 21	100%	33%
Day 42	100%	83%
Degraded Veligers	**Blood and Tissue Kit**	**Soil Kit**
1 Degraded Veliger		
Day 2	100%	89%
Day 21	11%	0%
Day 42	78%	0%
25 Degraded Veligers		
Day 2	100%	94%
Day 21	39%	0%
Day 42	33%	22%

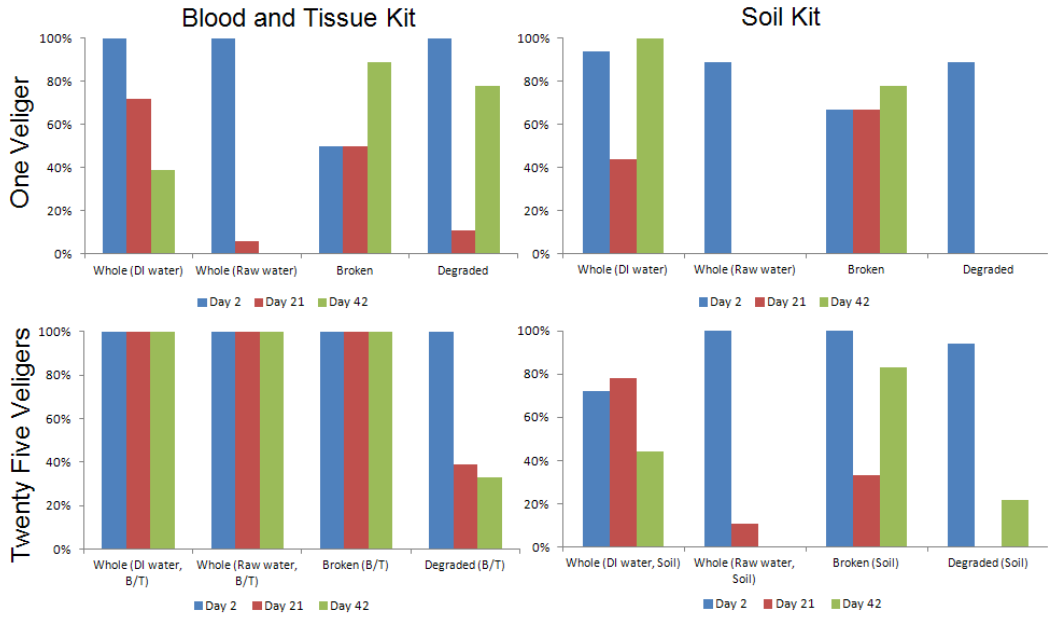

Figure 1: Results of the PCR analysis of a single veliger or twenty five veligers in DI or raw water, broken, or degraded over time (days 2, 21, and 42) with DNA extracted using either the blood and tissue kit or soil kit.

Blood and Tissue Kit and Soil Kit Analysis of dDNA

The blood and tissue kit was not as successful at detecting dDNA as iDNA (Table 2, Figure 2). Both the blood and tissue kit and the soil kit were relatively unsuccessful at detecting small concentrations (5 ng/µL) of dDNA. Other than the 67% positive achieved by the blood and tissue kit at day 2, all of the other PCR reactions were negative at the 5 ng/µL dDNA level. More positives were found at the 80 ng/µL dDNA level. The number of days to analysis did not appear to impact the blood and tissue kit results as positives were at 67% on day 2, 0% on day 21, and 100% on day 42. The number of days to analysis did appear to impact the results of the soil kit as detection decreased from 100% on day 2 to 39% and 17% on days 21 and 42 (Table 2, Figure 2).

Table 2: Percent of PCR positive results in samples containing 5 ng/µL or 80 ng/µL quagga mussel dDNA extracted with the blood and tissue kit and soil kit.

5 ng/µL DNA	**Blood and Tissue Kit**	**Soil Kit**
Day 2	67%	0%
Day 21	0%	0%
Day 42	0%	0%
80 ng/µL DNA	**Blood and Tissue Kit**	**Soil Kit**
Day 2	67%	100%
Day 21	0%	39%
Day 42	100%	17%

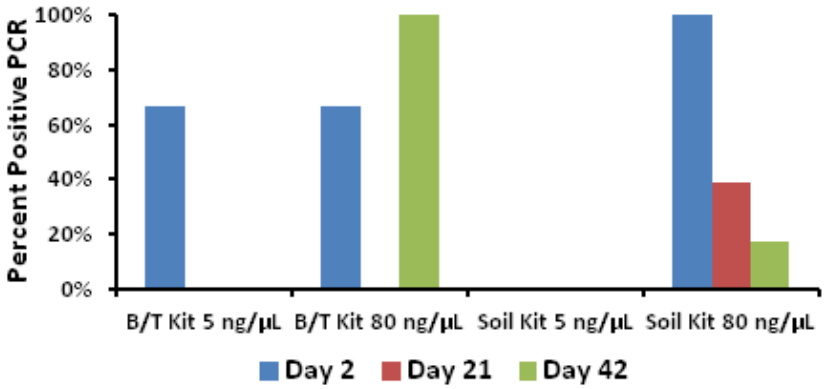

Figure 2: Percent of PCR positive results in samples with either 5 ng/µL or 80 ng/µL quagga mussel dDNA extracted with the blood and tissue (B/T) kit or soil kit.

Discussion and Conclusions

The goal of this study was to demonstrate how multiple factors (extraction kit type, amount of DNA in the sample, days to analysis, and inhibitors) impact PCR detection of quagga mussel DNA. An additional goal was to demonstrate that the PCR extraction methods used by RDLES are capable of detecting eDNA (both iDNA and dDNA) in early detection samples when veliger bodies are not found in the sample by microscopy.

In general, when more veligers are present in a sample a greater percent of samples will be positive over time. This result was expected because a greater amount of DNA is present in the sample. The blood and tissue kit appears to be more effective and consistent at detecting veligers. The soil kit did not perform as well; it is possible that not enough inhibitors (zooplankton) were added to be comparable to a raw water plankton tow sample. It may have been more effective and more comparable to real world samples if the zooplankton were centrifuged into a pellet prior to use. Without enough inhibitors to overcome the chemistry of the kit, the soil kit was no longer optimized, resulting in false negatives and inconsistent results. Other studies conducted by RDLES have shown that the soil kit outperformed the blood and tissue kit in overcoming inhibitors. The Qiagen FDA method for extracting DNA for barcoding samples is used for small sample volumes and would never be used for raw water pellets. In this study the blood and tissue method was used to test the kits ability to overcome inhibitors.

The number of days until analysis does not appear to be as critical in the detection of iDNA, if samples are preserved with alcohol and buffered. Free floating dDNA is not protected by a cell wall like iDNA, so it may be more susceptible to degradation over-time. The variability of the PCR results may also be due to the addition of raw water and zooplankton. The water chemistry and the additional non-target genetic material and humic acids may interfere with the PCR reactions.

DNA of whole veligers was detected at greater percentages in DI water when compared to raw water, especially at lower concentrations.

It was predicted that broken veligers would lose PCR signal faster than whole veligers or degraded veligers, considering the breaking action may damage the cell walls and expose the DNA. However, the degraded veligers lost PCR signal quicker for both extraction kits. These findings may have resulted from the degradation process. It is possible that tissue degradation occurred during exposure to the acetic acid rinse and not due to poor performance by the blood and tissue kit.

In this study, free floating dDNA was simulated by using DNA extracted from adults. This study shows that dDNA can be detected by the PCR methods utilized by RIDLES; however the results are inconsistent and not reproducible at either the 5 ng/µl or the 80 ng/µL concentration. While the likelihood of detecting dDNA increased when 80 ng/µL of free floating DNA was inserted into the sample, it is still likely to get false negative results with either DNA extraction method. The concentration of DNA in one veliger or one adult is still unknown. More research needs to be done in this area to determine how many veligers it requires to get a DNA concentration equal to 5 ng/µL. It is possible that 5 ng/µL is more DNA than could ever be in a single veliger.

The average time for sample analysis at RDLES is 21 days (Carmon, Keele, Pucherelli, & Hosler, 2014). Therefore, if dDNA is present in a raw water sample the likelihood of achieving a positive PCR result would be unlikely at this time point unless 80 ng/µL, or more, quagga mussel DNA is collected in the extraction. Smaller concentrations of dDNA will have likely degraded by the time the sample is analyzed by microscopy and PCR.

Overall, the results of this study indicate that it is possible to achieve a positive PCR result on a water sample that was found to be negative by microscopy, because PCR can detect free floating dDNA and veligers that are degraded and broken apart. Unfortunately, many of the PCR results in this study were negative even though a known source of DNA was present in the sample. These results suggest that early detection PCR is likely to produce more false negatives than false positives. More research needs to be done to determine how much DNA is in one veliger, and more importantly on how water quality and chemistry affects the DNA extraction chemistries of the blood and tissue kit, and the soil kit. While microscopy is still an import aspect of dreissenid early detection, this study indicates that PCR testing of dreissenid early detection samples is a valuable tool that is capable of detecting signs of dreissenid presence that would otherwise be missed by microscopy alone.

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Appendices

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
2	Control	2	1	Qiagen	1	1	1	1					1.00
3	Control	2	2	Qiagen	1	1	1	1					1.00
4	Control	2	4	Qiagen	1	1	1	1					1.00
5	Control	2	5	Qiagen	25	1	1	1					1.00
6	Control	2	6	Qiagen	25	1	1	1					1.00
7	Control	2	8	Qiagen	25	1	1	1					1.00
8	Control	2	9	Soil	1	1	1	1					1.00
9	Control	2	10	Soil	1	0	1	1					0.67
10	Control	2	12	Soil	1	1	1	1					1.00
11	Control	2	13	Soil	25	1	1	1					1.00
12	Control	2	14	Soil	25	1	1	1					1.00
13	Control	2	16	Soil	25	1	1	1					1.00
14	Degraded	2	1	Qiagen	1	1	1	1					1.00
15	Degraded	2	2	Qiagen	1	1	1	1					1.00
16	Degraded	2	4	Qiagen	1	1	1	1					1.00
17	Degraded	2	5	Qiagen	25	1	1	1					1.00
18	Degraded	2	6	Qiagen	25	1	1	1					1.00
19	Degraded	2	8	Qiagen	25	1	1	1					1.00
20	Degraded	2	9	Soil	1	1	1	0	1	1	1		0.83
21	Degraded	2	10	Soil	1	1	0	1	1	1	1		0.83
22	Degraded	2	12	Soil	1	1	1	1					1.00
23	Degraded	2	13	Soil	25	1	0	1	1	1	1		0.83
24	Degraded	2	14	Soil	25	1	1	1					1.00
25	Degraded	2	16	Soil	25	1	1	1					1.00
26	Broken	2	1	Qiagen	1	1	1	1					1.00
27	Broken	2	2	Qiagen	1	0	0	0	0	0	1		0.17
28	Broken	2	4	Qiagen	1	1	0	0					0.33
29	Broken	2	5	Qiagen	25	1	1	1					1.00
30	Broken	2	6	Qiagen	25	1	1	1					1.00

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
31	Broken	2	8	Qiagen	25	1	1	1					1.00
32	Broken	2	9	Soil	1	1	1	1					1.00
33	Broken	2	10	Soil	1	0	0	0					0.00
34	Broken	2	12	Soil	1	1	1	1					1.00
35	Broken	2	13	Soil	25	1	1	1					1.00
36	Broken	2	14	Soil	25	1	1	1					1.00
37	Broken	2	16	Soil	25	1	1	1					1.00
38	Cont+DI	2	1	Qiagen	1	1	1	1					1.00
39	Cont+DI	2	2	Qiagen	1	1	1	1					1.00
40	Cont+DI	2	4	Qiagen	1	1	1	1					1.00
41	Cont+DI	2	5	Qiagen	25	1	1	1					1.00
42	Cont+DI	2	6	Qiagen	25	1	1	1					1.00
43	Cont+DI	2	8	Qiagen	25	1	1	1					1.00
44	Cont+DI	2	9	Soil	1	1	0	1	1	1	1		0.83
45	Cont+DI	2	10	Soil	1	1	1	1					1.00
46	Cont+DI	2	12	Soil	1	1	1	1					1.00
47	Cont+DI	2	13	Soil	25	1	1	1					1.00
48	Cont+DI	2	14	Soil	25	0	0	0	0	1	0		0.17
49	Cont+DI	2	16	Soil	25	1	1	1					1.00
50	Control	21	1	Qiagen	1	0	0	0	0	1	0		0.17
51	Control	21	2	Qiagen	1	0	0	0	0	0	0		0.00
52	Control	21	4	Qiagen	1	0	0	0	0	0	0		0.00
53	Control	21	5	Qiagen	25	1	1	1					1.00
54	Control	21	6	Qiagen	25	1	1	1					1.00
55	Control	21	8	Qiagen	25	1	1	1					1.00
56	Control	21	9	Soil	1	0	0	0	0	0	0		0.00
57	Control	21	10	Soil	1	0	0	0	0	0	0		0.00
58	Control	21	12	Soil	1	0	0	0	0	0	0		0.00
59	Control	21	13	Soil	25	0	0	0	1	1	0		0.33

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
60	Control	21	14	Soil	25	0	0	0	0	0	0		0.00
61	Control	21	16	Soil	25	0	0	0	0	0	0		0.00
62	Degraded	21	1	Qiagen	1	1	0	0	0	0	0		0.17
63	Degraded	21	2	Qiagen	1	1	0	0	0	0	0		0.17
64	Degraded	21	4	Qiagen	1	0	0	0	0	0	0		0.00
65	Degraded	21	5	Qiagen	25	0	0	0	0	0	0		0.00
66	Degraded	21	6	Qiagen	25	1	1	1	1	1	1		1.00
67	Degraded	21	8	Qiagen	25	0	0	0	1	0	0		0.17
68	Degraded	21	9	Soil	1	0	0	0	0	0	0		0.00
69	Degraded	21	10	Soil	1	0	0	0	0	0	0		0.00
70	Degraded	21	12	Soil	1	0	0	0	0	0	0		0.00
71	Degraded	21	13	Soil	25	0	0	0	0	0	0		0.00
72	Degraded	21	14	Soil	25	0	0	0	0	0	0		0.00
73	Degraded	21	16	Soil	25	0	0	0	0	0	0		0.00
74	Broken	21	1	Qiagen	1	0	0	0	1	1	1		0.50
75	Broken	21	2	Qiagen	1	0	0	0	1	1	1		0.50
76	Broken	21	4	Qiagen	1	0	0	0	1	1	1		0.50
77	Broken	21	5	Qiagen	25	1	1	1	1	1	1		1.00
78	Broken	21	6	Qiagen	25	1	1	1	1	1	1		1.00
79	Broken	21	8	Qiagen	25	1	1	1	1	1	1		1.00
80	Broken	21	9	Soil	1	1	0	0	0	0	0		0.17
81	Broken	21	10	Soil	1	1	1	0	1	1	1		0.83
82	Broken	21	12	Soil	1	1	1	1	1	1	1		1.00
83	Broken	21	13	Soil	25	0	1	1	0	0	0		0.33
84	Broken	21	14	Soil	25	1	1	1	0	0	0		0.50
85	Broken	21	16	Soil	25	0	1	0	0	0	0		0.17
86	Cont+DI	21	1	Qiagen	1	0	1	1	1	1	1		0.83
87	Cont+DI	21	2	Qiagen	1	0	0	0	1	1	1		0.50
88	Cont+DI	21	4	Qiagen	1	1	1	0	1	1	1		0.83

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
88	Cont+DI	21	4	Qiagen	1	1	1	0	1	1	1		0.83
89	Cont+DI	21	5	Qiagen	25	1	1	1					1.00
90	Cont+DI	21	6	Qiagen	25	1	1	1					1.00
91	Cont+DI	21	8	Qiagen	25	1	1	1					1.00
92	Cont+DI	21	9	Soil	1	0	0	0	1	1	1		0.50
93	Cont+DI	21	10	Soil	1	0	0	0	1	1	1		0.50
94	Cont+DI	21	12	Soil	1	0	0	0	1	1	0		0.33
95	Cont+DI	21	13	Soil	25	1	1	1	1	1	0		0.83
96	Cont+DI	21	14	Soil	25	1	1	0	1	1	1		0.83
97	Cont+DI	21	16	Soil	25	0	1	0	1	1	1		0.67
98	Control	42	1	Qiagen	1	0	0	0	0	0	0		0.00
99	Control	42	2	Qiagen	1	0	0	0	0	0	0		0.00
100	Control	42	4	Qiagen	1	0	0	0	0	0	0		0.00
101	Control	42	5	Qiagen	25	1	1	1					1.00
102	Control	42	6	Qiagen	25	1	1	1					1.00
103	Control	42	8	Qiagen	25	1	1	1					1.00
104	Control	42	9	Soil	1	0	0	0	0	0	0		0.00
105	Control	42	10	Soil	1	0	0	0	0	0	0		0.00
106	Control	42	12	Soil	1	0	0	0	0	0	0		0.00
107	Control	42	13	Soil	25	0	0	0	0	0	0		0.00
108	Control	42	14	Soil	25	0	0	0	0	0	0		0.00
109	Control	42	16	Soil	25	0	0	0	0	0	0		0.00
110	Degraded	42	1	Qiagen	1	1	1	1	1	1	1		1.00
111	Degraded	42	2	Qiagen	1	1	1	0	0	1	1		0.67
112	Degraded	42	4	Qiagen	1	1	0	0	1	1	1		0.67
113	Degraded	42	5	Qiagen	25	0	0	0	0	0	0		0.00
114	Degraded	42	6	Qiagen	25	1	1	1	0	0	0		0.50
115	Degraded	42	8	Qiagen	25	1	1	1	0	0	0		0.50
116	Degraded	42	9	Soil	1	0	0	0	0	0	0		0.00

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
117	Degraded	42	10	Soil	1	0	0	0	0	0	0		0.00
118	Degraded	42	12	Soil	1	0	0	0	0	0	0		0.00
119	Degraded	42	13	Soil	25	0	0	0	1	0	0		0.17
120	Degraded	42	14	Soil	25	0	0	0	0	0	0		0.00
121	Degraded	42	16	Soil	25	1	1	1	0	0	0		0.50
122	Broken	42	1	Qiagen	1	1	1	1	1	1	1		1.00
123	Broken	42	2	Qiagen	1	1	1	1	1	1	1		1.00
124	Broken	42	4	Qiagen	1	1	0	0	1	1	1		0.67
125	Broken	42	5	Qiagen	25	1	1	1					1.00
126	Broken	42	6	Qiagen	25	1	1	1					1.00
127	Broken	42	8	Qiagen	25	1	1	1					1.00
128	Broken	42	9	Soil	1	0	0	0	1	1	1		0.50
129	Broken	42	10	Soil	1	1	1	1	1	1	1		1.00
130	Broken	42	12	Soil	1	0	1	1	1	1	1		0.83
131	Broken	42	13	Soil	25	1	1	1	1	1	1		1.00
132	Broken	42	14	Soil	25	1	1	1	1	1	1		1.00
133	Broken	42	16	Soil	25	0	0	0	1	1	1		0.50
134	Cont+DI	42	1	Qiagen	1	1	1	1	0	0	0		0.50
135	Cont+DI	42	2	Qiagen	1	1	1	1	0	0	0		0.50
136	Cont+DI	42	4	Qiagen	1	1	0	0	0	0	0		0.17
137	Cont+DI	42	5	Qiagen	25	1	1	1	1	1	1		1.00
138	Cont+DI	42	6	Qiagen	25	1	1	1	1	1	1		1.00
139	Cont+DI	42	8	Qiagen	25	1	1	1	1	1	1		1.00
140	Cont+DI	42	9	Soil	1	1	1	1					1.00
141	Cont+DI	42	10	Soil	1	1	1	1					1.00
142	Cont+DI	42	12	Soil	1	1	1	1					1.00
143	Cont+DI	42	13	Soil	25	1	0	1	0	0	0		0.33
144	Cont+DI	42	14	Soil	25	1	1	1	0	0	0		0.50
145	Cont+DI	42	16	Soil	25	1	1	1	0	0	0		0.50

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
147	Control (Blank)	2	3	Qiagen	1	0	0	0					0.00
148	Control (Blank)	2	7	Qiagen	25	0	0	0					0.00
149	Control (Blank)	2	11	Soil	1	0	0	0					0.00
150	Control (Blank)	2	15	Soil	25	0	0	0					0.00
151	Degraded (Blank)	2	3	Qiagen	1	0	0	0					0.00
152	Degraded (Blank)	2	7	Qiagen	25	0	0	0					0.00
153	Degraded (Blank)	2	11	Soil	1	0	0	0					0.00
154	Degraded (Blank)	2	15	Soil	25	0	0	0	0	0	0		0.00
155	Broken (Blank)	2	3	Qiagen	1	0	0	0					0.00
156	Broken (Blank)	2	7	Qiagen	25	0	0	0					0.00
157	Broken (Blank)	2	11	Soil	1	0	0	0					0.00
158	Broken (Blank)	2	15	Soil	25	0	0	0					0.00
159	Cont+DI (Blank)	2	3	Qiagen	1	0	0	0					0.00
160	Cont+DI (Blank)	2	7	Qiagen	25	0	0	0					0.00
161	Cont+DI (Blank)	2	11	Soil	1	0	0	0					0.00
162	Cont+DI (Blank)	2	15	Soil	25	0	0	0					0.00
163	Control (Blank)	21	3	Qiagen	1	0	0	0	0	0	0		0.00
164	Control (Blank)	21	7	Qiagen	25	0	0	0					0.00
165	Control (Blank)	21	11	Soil	1	0	0	0	0	0	0		0.00
166	Control (Blank)	21	15	Soil	25	0	0	0	0	0	0		0.00
167	Degraded (Blank)	21	3	Qiagen	1	0	0	0	0	0	0		0.00
168	Degraded (Blank)	21	7	Qiagen	25	0	0	0	0	0	0		0.00
169	Degraded (Blank)	21	11	Soil	1	0	0	0	0	0	0		0.00
170	Degraded (Blank)	21	15	Soil	25	0	0	0	0	0	0		0.00
171	Broken (Blank)	21	3	Qiagen	1	0	0	0	0	0	0		0.00
172	Broken (Blank)	21	7	Qiagen	25	0	0	0	0	0	0		0.00
173	Broken (Blank)	21	11	Soil	1	0	0	0	0	0	0		0.00
174	Broken (Blank)	21	15	Soil	25	0	0	0	0	0	0		0.00
175	Cont+DI (Blank)	21	3	Qiagen	1	0	0	0	0	0	0		0.00

	A	B	C	D	E	F	G	H	I	J	K	L	M
1	Prep	Day	Sample #	Extract	# of veliger	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
175	Cont+DI (Blank)	21	3	Qiagen	1	0	0	0	0	0	0		0.00
176	Cont+DI (Blank)	21	7	Qiagen	25	0	0	0					0.00
177	Cont+DI (Blank)	21	11	Soil	1	0	0	0	0	0	0		0.00
178	Cont+DI (Blank)	21	15	Soil	25	0	0	0	0	0	0		0.00
179	Control (Blank)	42	3	Qiagen	1	0	0	0	0	0	0		0.00
180	Control (Blank)	42	7	Qiagen	25	0	0	0					0.00
181	Control (Blank)	42	11	Soil	1	0	0	0	0	0	0		0.00
182	Control (Blank)	42	15	Soil	25	0	0	0	0	0	0		0.00
183	Degraded (Blank)	42	3	Qiagen	1	0	0	0	0	0	0		0.00
184	Degraded (Blank)	42	7	Qiagen	25	0	0	0	0	0	0		0.00
185	Degraded (Blank)	42	11	Soil	1	0	0	0	0	0	0		0.00
186	Degraded (Blank)	42	15	Soil	25	0	0	0	0	0	0		0.00
187	Broken (Blank)	42	3	Qiagen	1	0	0	0	0	0	0		0.00
188	Broken (Blank)	42	7	Qiagen	25	0	0	0					0.00
189	Broken (Blank)	42	11	Soil	1	0	0	0	0	0	0		0.00
190	Broken (Blank)	42	15	Soil	25	0	0	0	0	0	0		0.00
191	Cont+DI (Blank)	42	3	Qiagen	1	0	0	0	0	0	0		0.00
192	Cont+DI (Blank)	42	7	Qiagen	25	0	0	0	0	0	0		0.00
193	Cont+DI (Blank)	42	11	Soil	1	0	0	0					0.00
194	Cont+DI (Blank)	42	15	Soil	25	0	0	0	0	0	0		0.00

	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
1		Prep	Day	Sample #	Extract	amt of DNA ng/ul	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
2		DNA	2	1	Qiagen	5	1	1	1					1.00
3		DNA	2	2	Qiagen	5	1	1	1					1.00
4		DNA (Blank)	2	3	Qiagen	5	0	0	0					0.00
5		DNA	2	4	Qiagen	5	0	0	0	0	0	0		0.00
6		DNA	2	5	Qiagen	80	0	1	0	0	1	0		0.33
7		DNA	2	6	Qiagen	80	1	1	1					1.00
8		DNA (Blank)	2	7	Qiagen	80	0	0	0					0.00
9		DNA	2	8	Qiagen	80	0	0	1	1	1	1		0.67
10		DNA	2	9	Soil	5	0	0	0					0.00
11		DNA	2	10	Soil	5	0	0	0					0.00
12		DNA (Blank)	2	11	Soil	5	0	0	0					0.00
13		DNA	2	12	Soil	5	0	0	0					0.00
14		DNA	2	13	Soil	80	1	1	1					1.00
15		DNA	2	14	Soil	80	1	1	1					1.00
16		DNA (Blank)	2	15	Soil	80	0	0	0					0.00
17		DNA	2	16	Soil	80	1	1	1					1.00
18		DNA	21	1	Qiagen	5	0	0	0	0	0	0		0.00
19		DNA	21	2	Qiagen	5	0	0	0	0	0	0		0.00
20		DNA (Blank)	21	3	Qiagen	5	0	0	0	0	0	0		0.00
21		DNA	21	4	Qiagen	5	0	0	0	0	0	0		0.00
22		DNA	21	5	Qiagen	80	0	0	0	0	0	0		0.00
23		DNA	21	6	Qiagen	80	0	0	0	0	0	0		0.00
24		DNA (Blank)	21	7	Qiagen	80	0	0	0	0	0	0		0.00
25		DNA	21	8	Qiagen	80	0	0	0	0	0	0		0.00
26		DNA	21	9	Soil	5	0	0	0	0	0	0		0.00
27		DNA	21	10	Soil	5	0	0	0	0	0	0		0.00
28		DNA (Blank)	21	11	Soil	5	0	0	0	0	0	0		0.00
29		DNA	21	12	Soil	5	0	0	0	0	0	0		0.00
30		DNA	21	13	Soil	80	1	1	0	0	0	0		0.33

	P	Q	R	S	T	U	V	W	X	Y	Z	AA	AB	AC
1		Prep	Day	Sample #	Extract	amt of DNA ng/ul	PCR Rep 1	PCR Rep 2	PCR Rep 3	PCR Rep 4	PCR Rep 5	PCR Rep 6	amt DNA in sample (spec)	Average PCR Success
31		DNA	21	14	Soil	80	1	1	1	0	0	0		0.50
32		DNA (Blank)	21	15	Soil	80	0	0	0	0	0	0		0.00
33		DNA	21	16	Soil	80	1	1	0	0	0	0		0.33
34		DNA	42	1	Qiagen	5	0	0	0	0	0	0		0.00
35		DNA	42	2	Qiagen	5	0	0	0	0	0	0		0.00
36		DNA (Blank)	42	3	Qiagen	5	0	0	0	0	0	0		0.00
37		DNA	42	4	Qiagen	5	0	0	0	0	0	0		0.00
38		DNA	42	5	Qiagen	80	1	1	1					1.00
39		DNA	42	6	Qiagen	80	1	1	1					1.00
40		DNA (Blank)	42	7	Qiagen	80	0	0	0					0.00
41		DNA	42	8	Qiagen	80	1	1	1					1.00
42		DNA	42	9	Soil	5	0	0	0	0	0	0		0.00
43		DNA	42	10	Soil	5	0	0	0	1	1	1		0.50
44		DNA (Blank)	42	11	Soil	5	0	0	0	0	0	0		0.00
45		DNA	42	12	Soil	5	0	0	0	0	0	0		0.00
46		DNA	42	13	Soil	80	0	0	0	0	0	0		0.00
47		DNA	42	14	Soil	80	0	0	0	1	1	1		0.50
48		DNA (Blank)	42	15	Soil	80	0	0	0	0	0	0		0.00
49		DNA	42	16	Soil	80	0	0	0	0	0	0		0.00

Share drive folder, name and path where data are stored:

H:/EnvRes Share/Mussel Samples/2014 Research Proposals/eDNA

Points of Contact:

Denise Hosler: dhosler@usbr.gov (303-445-2195)
Sherri Pucherelli: spucherelli@usbr.gov (303-445-2015)
Jacque Keele:jkeele@usbr.gov (303-445-2187)