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Analysis of environmental DNA from sediments for detection of invasive dreissenid mussels

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The present study evaluated the use of sediment DNA analysis for detection of quagga mussels from environmental samples. The study evaluated techniques for sample preservation and DNA extraction, and developed methods suited to the sample types encountered in Reclamation reservoirs. Established quantitative PCR (qPCR) assays were successfully used to detect quagga mussel DNA in sediments from reservoirs and lakes with established populations. The sediment eDNA technique was validated, but its applicability to early detection of invasive mussels may be limited.

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Acronyms and Abbreviations

Cq	Quantification cycle
EcoLab	Ecological Research Laboratory
eDNA	Environmental DNA
qPCR	Quantitative polymerase chain reaction
Reclamation	Bureau of Reclamation

Measurements

°C	degree Celsius
ml	milliliter

Contents

Executive Summary

Environmental DNA (eDNA) has become an important tool for early detection of invasive species. In Reclamation's Ecological Research Laboratory (EcoLab) eDNA analysis is regularly used for early detection of invasive quagga and zebra mussels. eDNA analysis is a rapidly developing field, and new techniques and assays are regularly being introduced. One novel methodology is the use of sediment eDNA analysis, which may provide an additional approach for early detection of invasive species. eDNA has been shown to be more concentrated in sediments than in overly waters for a number of aquatic species, suggesting that this technique could provide greater sensitivity for the detection of species of interest.

The present study evaluated the use of sediment eDNA analysis for detection of DNA from quagga mussels. Methods for sample preservation and DNA extraction from sediment samples were evaluated and refined. Samples were tested from six reservoirs and lakes with established quagga mussel populations. Quagga mussel DNA was successfully detected from five of the six sites. The one site where quagga mussel eDNA was not detected in sediments tends to have low population numbers based on surveys of veliger numbers. The sediment eDNA technique was successfully established in the EcoLab, but its applicability to early detection of invasive mussels may be limited, as small incipient populations may not deposit sufficient DNA in sediments for reliable detection.

Introduction

Analysis of environmental DNA (eDNA) is based on detection of DNA for species of interest from samples collected from the environment (e.g., water or soil) rather than from an individual's tissue. Over the last 15 years eDNA has been developed as a technique for early detection of invasive species. Incipient populations of invasive species can often be small and difficult to detect by traditional survey methods. eDNA provides an additional tool for early detection and can provide evidence for the presence of a species in a new environment before individuals are identified. Reclamation's Ecological Research Laboratory (EcoLab) uses eDNA analysis to monitor for early detection of dreissenid mussels ((quagga mussel (*Dreissena rostriformis bugensis*) and zebra mussel (*Dreissena polymorpha*)), providing a complement to traditional microcropy based techniques for identifying mussel veligers. These two techniques have been extremely valuable, but additional techniques could further improve early detection efforts.

Most eDNA studies of aquatic species, including those for quagga and zebra mussels, have focused on the analysis of water samples. However, there has been investigation of using eDNA analysis of sediment samples as another tool for study the distribution of organisms of interest. One important characteristic of DNA deposited in sediments is that it tends to degrade more slowly than DNA suspended in the water column (Nielsen et al., 2007). Because of this, much of the work on sediment eDNA has been from the perspective of paleogenetics, with studies investigating historical changes in populations. eDNA also tends to be more concentrated in sediments than in the overlying water (Turner et al., 205), suggesting that sediment eDNA analysis could increase the sensitivity of early detection efforts focused on species at low abundance. Recently a number of studies have evaluated the potential to use sediment eDNA for early detection of invasive species (Azis et al., 2020, Crane et al., 2021). This approach has shown promise, but to date sediment eDNA analysis has not been applied to early detection of quagga and zebra mussels.

The present study focused on evaluating the use of sediment eDNA analysis for detection of quagga mussel DNA. Techniques for sample preservation and DNA extraction were tested to determine the optimal methods for quagga mussel DNA recovery. Samples were collected from Reclamation reservoirs and were tested using a species-specific assay for quagga mussel. Samples were also collected and analyzed from Lake Michigan, which has a large quagga mussel population, but which historically was dominated by zebra mussels.

Methods

Sample collection

Sampling was performed by collecting sediment from below the waterline at all reservoirs and lakes. Samples from sites in the Western United States were collected with an Ekman grab sampler, or were scooped with a sterile cup. Samples from Lake Michigan were collected with a Ponar grab sampler. Approximately 10 ml of sediment was transferred to a sterile 50-ml conical tube using a sterile spatula. Excess water was decanted from samples, and 30 ml either ethanol or Longmire's solution was added to the remaining sediment as a preservative. Field blanks containing commercial potting soil and preservative were prepared onsite prior to sample collection. Sample were collected from Lake Mead, Lake Powell, Theodore Roosevelt Lake, Apache Lake, Canyon Lake, Saguaro Lake, and Lake Michigan. Collection site coordinates are listed in Table 1.

Table 1. Sampling locations and geographic coordinates

Sample location	State	Latitude	Longitude
Lake Mead	NV-AZ	36.0291	-114.7722
Lake Powell	UT-AZ	36.9944	-111.4831
Theodore Roosevelt Lake	AZ	33.6731	-111.1319
Apache Lake	AZ	33.6543	-111.1854
Canyon Lake	AZ	33.5378	-111.4293
Saguaro Lake	AZ	33.5758	-111.5362
Lake Michigan	MI	43.2045	-86.5677

Analysis

DNA was extracted using the *Quick*-DNA™ Fecal/Soil Microbe Microprep Kit (Zymo Research Corporation) or the DNeasy PowerSoil Pro Kit (QIAGEN, Inc.) following the manufacturers' protocols. Quantitative polymerase chain reaction assays (qPCR) were performed using PerfeCTa qPCR ToughMix (Quantbio) and the quagga mussel-specific primers and probe QMCOI (Sepulveda et al., 2019) or the zebra mussel-specific primers and probe ZEBCOI (Gingera et al., 2017). For replicate reactions were tested for each sample. Thermal cycling was performed using a BioRad CFX96, with Maestro software (Bio-Rad Laboratories, Inc.) used for instrument control and data analysis. A 2-step thermal cycling protocol was performed, with an initial denaturation step 95°C for 3 minutes, followed by 40 cycles of 95°C for 15 seconds and 60°C for 1 minute. Samples were scored as positive if the Cq value was <= 38 cycles. A gBlock synthetic oligonucleotide, run in a dilution series ranging from 2×10^0 to 2×10^5 copies per reaction, was included on each qPCR analysis plate as a positive control. Negative control samples (blanks) were generated at each step of sample collection and processing to test for potential contamination. There controls including field blanks, DNA extraction blanks, and qPCR reaction no-template controls. All controls were tested by qPCR. All reactions also included TaqMan Exogenous Internal Positive Control (IPC) reagent

(Thermo Fisher Scientific) to test for any reaction inhibition. TaqMan Exogenous IPC was used at 1/5 of the manufacturer's recommended concentration.

Results

DNA extraction and preservatives

Two preservatives, ethanol and Longmire's solution, were tested for their ability to maintain quagga mussel DNA is sediment samples. Samples were collected from three reservoirs where quagga mussels are known to be established, Lake Mead, Apache Lake, and Canyon Lake, and split between the two preservatives. Quagga mussel *COI* gene was detected from samples preserved with Longmire's solution from all three sites (Table *2*). Samples preserved in ethanol did not result in positive detections form any of the tested locations. Longmire's solution was used for all subsequent sample collections.

Two different kits for DNA extraction, *Quick*-DNA™ Fecal/Soil Microbe Microprep Kit (Zymo Research Corporation) and DNeasy PowerSoil Pro Kit (QIAGEN, Inc.), were also tested for their efficacy. DNA from the DNeasy PowerSoil Pro Kit resulted in positive detections when used with samples preserved in Longmire's solution (Table *2*). The *Quick*-DNA™ Fecal/Soil Microbe Microprep Kit did not result in detections when tested with samples preserved in either Longmire's solution or ethanol. The DNeasy PowerSoil Pro Kit was used for all subsequent DNA extractions.

During sample collection and processing it was noted that sediments from sampled waterbodies in the Western U.S. were very sandy, with large average sediments sizes. Directly processing such samples for DNA extraction without further processing resulted in low rates of positive detections, even from sites with established populations of quagga mussels. Sample processing was modified to capture smaller sediment size fractions within the sample. To accomplish this, samples were inverted to resuspend the sediment sample in the preservative. Samples were settled for a minute following inversion, allowing large sand to fall to the bottom of the collection tube. The supernatant containing preservative and suspended sediment was transferred to new 50-ml conical tube and centrifuged at 4000 rpm for 10 minutes to collect sediment for DNA extraction. This modification to the sample processing was used for all subsequent DNA extractions.

Table 2. qPCR detections from tested preservatives and DNA extraction kits

Sample location	Preservative	Extraction kit	Detection
Lake Mead	Ethanol	QuickDNA Fecal/Soil	Negative
Lake Mead	Ethanol	PowerSoil Pro	Negative
Lake Mead	Longmire's	QuickDNA Fecal/Soil	Negative
Lake Mead	Longmire's	PowerSoil Pro	Positive
Apache Lake	Ethanol	PowerSoil Pro	Negative
Apache Lake	Longmire's	PowerSoil Pro	Positive
Canyon Lake	Ethanol	PowerSoil Pro	Negative
Canyon Lake	Longmire's	PowerSoil Pro	Positive

Quagga mussel DNA detection

Sediment samples from seven waterbodies were tested for the quagga mussel *COI* gene by qPCR using the preservation and DNA extraction methods discussed above. Six of the waterbodies tested are known to have established quagga mussel populations. Of these six waterbodies with quagga mussels, sediment samples from five resulted in positive detections of the quagga *COI* gene (Table *3*). The one waterbody with a known quagga mussel population that did not result in a positive detection by qPCR was Saguaro Lake. Theodore Roosevelt Lake, which does not have an established quagga mussel population, was also tested and did not result in a detection of the quagga mussel *COI* gene by qPCR (Table *3*).

Table 3. Results for qPCR detection of quagga mussel COI gene

Sample location	Mussel status	Detection
Lake Mead	Established	Positive
Lake Powell	Established	Positive
Theodore Roosevelt Lake	Not established	Negative
Apache Lake	Established	Positive
Canyon Lake	Established	Positive
Saguaro Lake	Established	Negative
Lake Michigan	Established	Positive

Zebra mussel DNA detection

Sediment samples from Lake Michigan were also tested for zebra mussel DNA based on the fact that this species is also present in the Great Lakes. The zebra mussel *COI* gene was not detected in any of the samples from Lake Michigan.

Discussion

Sediment DNA recovery

For DNA extraction the present study tested two widely used commercial kits, the *Quick*-DNA™ Fecal/Soil Microbe Microprep and the DNeasy PowerSoil Pro Kit. Samples processed with the DNeasy PowerSoil Pro Kit reliably resulted in quagga mussel *COI* gene detections. In contrast, no detections were obtained from samples processed with the *Quick*-DNA™ Fecal/Soil Microbe Microprep. This kit is designed for soil and other samples with high levels of inhibitors and is regularly used by the EcoLab for processing tow net samples. It is not clear why the *Quick*-DNA™ Fecal/Soil Microbe Microprep did not produce any detections with the sediment samples in the current study. Analysis was also performed on the relative utility of two preservatives, ethanol and Longmire's solution. Samples preserved with Longmire's solution resulted in detection of the quagga *COI* gene when processed with the DNeasy PowerSoil Pro Kit, while samples preserved in ethanol did not produce detections after processing with either DNA extraction kit tested. In the current study preservation of sediment samples with Longmire's solution followed by DNA extraction with the DNeasy PowerSoil Pro Kit was found to provide an effective strategy for recovery of quagga mussel DNA.

It was also found that it is important to account for the physical characteristics of the sediment substrate being sampled. In the Western U.S. many sediments are sandy with large grain sizes, particularly in reservoirs along rivers with high sediment loads. eDNA appears to be concentrated in portions of the sediment with smaller grain sizes. This is likely due to both the available surface area in smaller sediment grains and the physiochemical characteristics of these grains. Selectively processing fractions of the sediment sample that had smaller grain sizes increased the rate of qPCR detection as compared with bulk processing of the sediment samples.

DNA detections

Quagga mussel DNA was successfully detected from nearly all the samples collected at sites with established populations. The one exception was Saguaro Lake is Arizona. Saquaro Lake is directly downstream of Apache Lake and Canyon Lake, which both had sediments with positive detections for the quagga mussel *COI* gene. These three reservoirs are regularly sampled for quagga mussel veligers, and Saquaro Lake tends to have lower veliger densities than do the other two reservoirs (data.usbr.gov). This could suggest that relatively large populations are needed for measurable quantities of DNA to be deposited and retained in sediments. However, additional sampling at this and other sites with low population numbers would be needed to validate this.

The fact that zebra mussel DNA was not detected from sediments in Lake Michigan could have several explanations. Zebra mussels where widespread in Lake Michigan in the 1990's and early 2000's, but their numbers have decreased over the last 20 years as quagga mussels have spread and become a dominant species. It is possible that insufficient DNA was deposited for detection, as historical populations densities of zebra mussels were not as high as the those of quagga mussels have become (Nalepa et al., 2017). It is also possible that zebra mussel DNA did not persists, or that

layers of sediment containing this DNA have subsequently been buried and was not captured during sample collection which was from close to the surface of the substrate.

The results of this study show that DNA from quagga mussel can be reliably detected from lakes and reservoirs with established populations. This provides an additional tool using eDNA to study populations of invasive and native species. However, the results of this project suggest that detection of DNA from dreissenid mussels in sediments may require a large, established population. Sediment eDNA analysis may therefore have limited utility for early detection of quagga and zebra mussels, as small incipient populations are unlikely to shed and deposit sufficient DNA into sediments for this approach to be reliable. Although it was outside the scope of the current project, sediment eDNA analysis may be most informative for retrospective studies where careful analysis of layers in sediment cores can provide information on historical trends in populations.

Data

Share Drive folder name and path where data are stored:

T:\Jobs\DO_NonFeature\Science and Technology\2020-PRG-Mussel Sediment Analysis

Point of Contact name, email, and phone:

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Final report (PDF)

Keywords:

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