

BAMBOO BROOK
170 LONGVIEW ROAD
FAR HILLS, NJ 07931
908-234-1225
908-234-1189 (FAX)
info@njconservation.org
www.njconservation.org



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Michele S. Byers

December 23, 2020

Ms. Maryann Carroll
Delaware River Greenway Partnership
PO Box 15
Stockton, New Jersey 08559

Dear Maryann:

In 2019, NJ Conservation and its project partners treated nine ponds in Raritan Township, New Jersey in order to eradicate a population of highly invasive Chinese pond mussel. This population is a significant concern because this species has proved to be very invasive in Europe, where it has spread through a number of major river systems and negatively impacted native mollusk species. The population is a direct threat to the Delaware River, as the ponds release water into the Wickecheoke Creek, which is a Delaware River tributary.

On behalf of the board and staff of New Jersey Conservation Foundation, thank you for the 2020 Wild and Scenic mini-grant award to collect and analyze eDNA samples in order to determine whether our 2019 eradication project was successful. The enclosed final report describes the activities we performed under this grant award.

Thank you again for your support. Please contact me at 908-234-1225 or Michele@njconservation.org if you need any additional information.

Sincerely,

Michele S. Byers
Executive Director



**Final Report to
Lower Delaware Wild and Scenic River
Protecting Water Quality in the Delaware River Watershed
eDNA Testing for the Highly Invasive Chinese Pond Mussel**

Project Summary

New Jersey Conservation Foundation (NJ Conservation), with funding from the Lower Delaware Wild and Scenic River Management Council, US National Park Service, and US Fish and Wildlife Service, contracted Rutgers University to collect water samples from a complex of ponds which was known to contain the only known population of invasive Chinese pond mussel (*Sinanodonta woodiana*) (CPM) in North America. Rutgers analyzed the samples using environmental DNA (eDNA) techniques to determine whether the CPM population was still extant following the 2019 eradication efforts. CPM eDNA was identified from two of the ponds sampled, indicating that a CPM population persists in the ponds and that additional eradication efforts are needed.

Background

The project site is 51-acre property in Franklin Township, Hunterdon County, NJ at the headwaters of the Wickecheoke Creek, approximately 12 miles upstream from the confluence with the Delaware River. There is a complex of nine man-made ponds on the property totaling about 4.4 acres in surface area. The ponds were constructed in the 1970s for the purpose of raising Asian carp. NJ Conservation acquired the site in 2007, and in 2010 carried out a project to eliminate the remaining carp. At that time, NJ Conservation discovered an unusually large freshwater mussel, which was later identified as Chinese pond mussel. CPM is known to be highly invasive in Europe, and is the only documented occurrence in all of North America.

In 2015, NJ Conservation and partners surveyed for CPM roughly half the length of the Wickecheoke Creek. Fortunately only a few shells and no live specimens were found. Also in 2015, the ponds were treated with Earthtec, a copper-based algacide in an attempt to eradicate the population. Copper is toxic to mussels, but only if maintained at a minimum effective level for an extended period of time through multiple treatments. At that time, Earthtec's label and New Jersey Pesticide Control regulations did not permit repeat applications. The single permitted treatment was not effective and live individuals were found subsequently. In the intervening years, Earthtec was relabeled as Earthtec QZ, and treatment protocols were developed to accommodate the repeated treatments needed to control mussels. This relabeling allowed NJ Conservation to resume efforts to eradicate the population.

In 2019, NJ Conservation, with support from US Fish and Wildlife Service, treated the ponds with Earthtec QZ, using a treatment protocol specifically designed to eliminate the CPM population. Based upon the intensity and duration of the 2019 treatment, it was believed that the CPM population had been destroyed. However, due to the depth of the ponds and the presumed small population size, specimens were extremely difficult to find. Therefore it was impossible to determine whether the 2019 treatment was effective by direct observation.

Project Description

In 2020, with support from the Lower Delaware Wild and Scenic River Management Council and US Fish and Wildlife Service, NJ Conservation contracted Rutgers University to collect water samples and analyze them for CPM eDNA. The team of researchers was led by Anthony Vastano and Julie Lockwood of Rutgers University's Department of Ecology, Evolution, and

Natural Resources. Field technicians visited the site to collect samples six times between August 11 and September 9, 2020. A total of 112 samples were collected, including a mix of surface water filter samples, grab bottle samples, and negative control samples (one per visit). Sampling was conducted at the beaver pond (WB), all five aquaculture ponds (W4 - W8), the outflow adjacent to W8, and in the creek where it crosses Allens Corner Road. Please see map below with approximate sampling locations (S= Surface sample, B = Bottle sample).

Originally, Rutgers planned to use the COI (Cytochrome oxidase I) region of the CPM genome to create an assay for detecting CPM eDNA. However, the COI region proved to have too much intraspecific genetic variation for the creation of a qPCR assay that would reliably detect CPM eDNA, based on sequences available within GenBank, the open access genetic sequence database maintained by the National Institutes of Health.

The Rutgers team explored other genetic regions (Internal transcribed spacer 1 and 2), and found that the ITS2 region was well conserved between sequences in GenBank. Tissue samples taken from the Chinese Pond Mussels collected at the NJ aquaculture ponds were secured from Dr. Arthur Bogan of the NC Museum of Natural Sciences. These samples were extracted and sequenced via Sanger sequencing along the ITS2 region with a custom designed set of CPM primers. Based on a consensus sequence between the NJ tissue samples and other CPM sequences found in GenBank, the Rutgers team created a custom qPCR assay that utilizes a molecular beacon probe that should have a high degree of specificity for CPM.

The custom assay was run on the collected samples, and positive results for presence of CPM eDNA was found in samples from two of the ponds, indicating a high likelihood that there is an extant population of CPM. For additional detail of methods used in sampling and analysis and the actual qPCR assay results, please see the attached report prepared by Vastano and Lockwood.

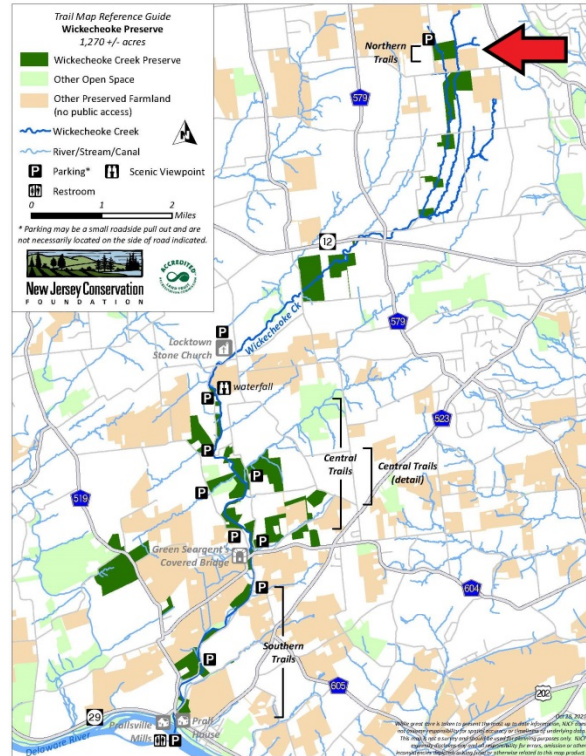
Next Steps

NJ Conservation will meet with biologists from US Fish and Wildlife Service, NJ Division of Fish and Wildlife, and the NJ Invasive Strike Team early in 2021 to determine next steps. It is anticipated that the next steps will include all of the following activities: 1. Visual surveying of ponds in which eDNA was detected to confirm an extant population, 2. Additional eDNA monitoring in future years, including monitoring downstream in the Wickecheoke Creek, and 3. Additional eradication efforts.

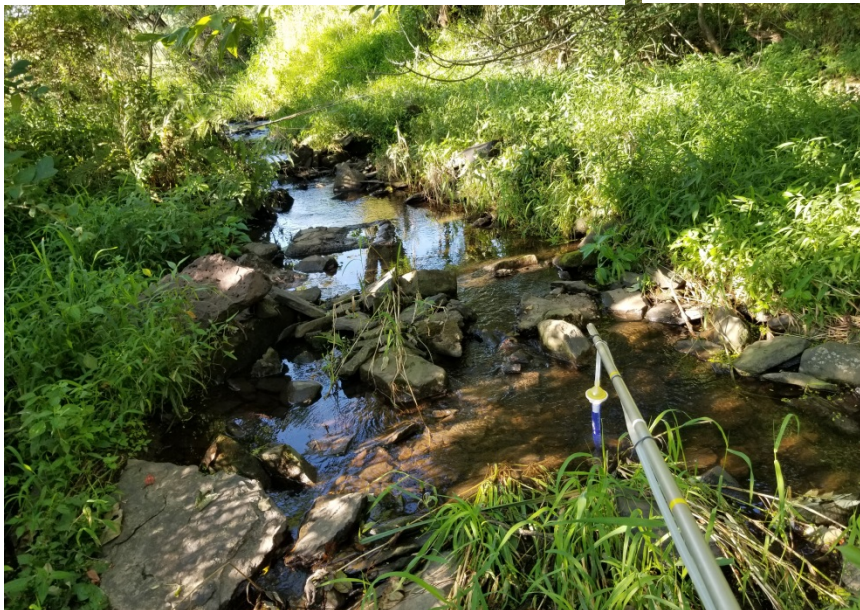
Photos



Typical Chinese Pond Mussel (*Sinodonta woodiana*)



Project location in Franklin Twp, Hunterdon County.



Collecting samples from pond outlet.



Collecting samples.



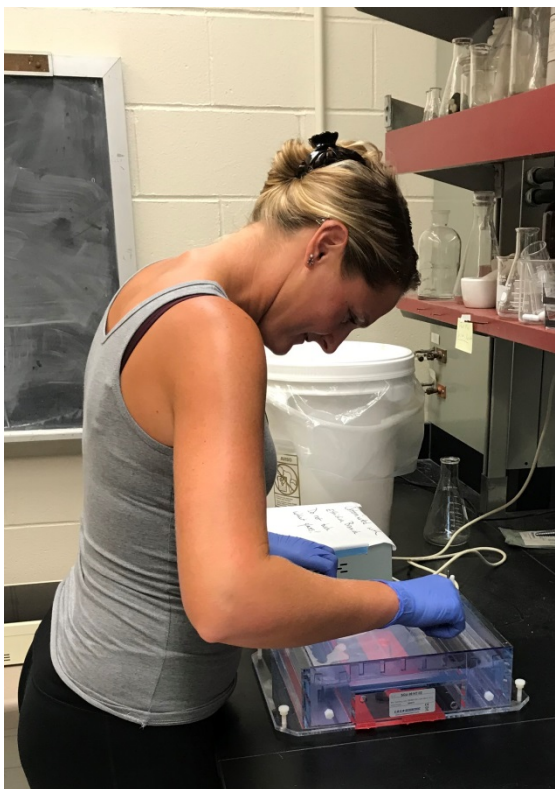
Processing samples in the field.



Sampling locations.



Processing samples in field.



Running qPCR analysis in laboratory.

Additional Funding

Additional funding in the amount of \$10,000 was secured from US Fish and Wildlife Service for the cost of collection and analysis of eDNA samples.

Unexpected Challenges

There were several unexpected challenges that came up during the course of the project. Several of these were directly related to the Covid-19 pandemic. At the time of planning this project and applying for funding, Covid-19 was known, but it was not yet known how significant and long-lasting its effects would be. Rutgers University, the contractor that performed both sampling collection and analysis was subject to severe work and travel restrictions for much of the project period. As a result, planning and contracting for this project, as well as sample collection, was delayed. In addition, the analysis technique used for the detecting Chinese Pond Mussel DNA was quantitative polymerase chain reaction (qPCR), the same technique used for many of the Covid-19 tests. As a result, equipment and materials needed to conduct the analysis were difficult to obtain and only available after significant delays.

An additional unexpected challenge was that the region of genetic material (Cytochrome Oxidase 1) previously used by researchers to identify mussels was found to contain too much intraspecific genetic variation to allow for the creation of a qPCR assay that would reliably detect Chinese Pond Mussel DNA. Therefore additional work was done to explore other DNA regions that could be used to create an assay that would provide more reliable, species specific results.

Unexpected Benefits

The Rutgers team was able to determine that the Internal Transcriber Spacer 2 (ITS2) region of genetic code contained much less intraspecific variation, and is therefore appropriate for use detecting this species' eDNA. This work will prove useful for detecting and managing this invasive species in both North American and Europe.

Description of Changes

Due to the Covid-19 delays described above samples were collected between August 11 and September 9, 2020, while original plans were to collect samples in June, July and August. The original budget itemized sampling and analysis supplies and equipment, but in practice these costs were all included in one contract fee to Rutgers University. Also, the final budget totaled \$1,354.36 less than the original total project cost. Lastly, the original plan was to sample all nine ponds on the property and the outlet from the neighbor's pond downstream. Due to budget constraints, the number of ponds sampled was reduced to six on the subject site plus the outlet of the neighbor's pond.

Publicity and Acknowledgements

Attached please find the final report from Rutgers University, acknowledging support from LDWS. Given the very recent completion of this project, we have no additional publicity to



Final Report to
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forward to you. Any pertinent materials will be forwarded to the LDWS as they become available, including NJ Conservation's 2020 Annual Report.

Project Budget

2020 Pond Mussel DNA project	Anticipated	Actual
Staff Time for Sampling	\$3,000	\$ 3,420.00
Travel	\$500	\$ 138.16
Professional Fees	-	\$12,961.30
50mL Conical Centrifuge Tubes (1 package of 300)	\$267.50	
500 mL PET Bottles, 16 oz. (108 @\$0.67)	\$72.36	
Cap 38/400 Pressure Sens Line (108 @ \$0.12)	\$12.96	
Quick DNA fecal/soil Kit (3 packages of 50 @ \$216/package)	\$648.00	
Primers and Probes	\$350	
qPCR Reagents (1 Rx, 5@\$129.60)	\$648.00	
Subcontracted Technician Labor for extractions and qPCR	\$3,200	
Subcontracted Labor for writing and analyses	\$9,175	
	\$17,873.82	\$16,519.46