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SPECIALIZING IN EDUCATION AND CONTROL

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eDNA survey for quagga mussels in Crystal Lake, Michigan
Final Report

Background

Quagga (*Dreissena rostriformis bugensis*) and zebra (*Dreissena polymorpha*) mussels are aquatic invasive species (AIS) that have caused costly economic and ecological damage in North America and Europe. Natives to western Asia, they have numerous characteristics that contribute to their invasive success, including microscopic floating larvae, ability to attach to many different substrates, and high tolerances for temperature variation, low oxygen, drying, and minimal food conditions.

Like many inland lakes, Crystal Lake has a well-established population of zebra mussels. We find young zebra mussels attached to many snails that we collect. Quagga mussels have followed zebra mussels invasions in many places in the Great Lakes region, and the Crystal Lake Watershed Association (CLWA) is concerned that quagga mussels may have arrived. Quagga mussels can have an even stronger ecological effect than the zebra mussel due to a higher water filtering capacity and a better ability to colonize soft substrates. Early detection is critical to decision making, monitoring associated changes in the lake, and any prospects for control.

Environmental DNA (eDNA) is a term used to describe genetic material deposited or shed into the environment by living organisms and can include both extracellular and intracellular DNA (the latter is cells shed from the organism). These materials can be free-floating in the water but also adhere to other microscopic organic material. The collection and isolation of eDNA from the environment has enabled an array of techniques for monitoring rare species, documenting biodiversity, and detecting invasive species like quagga and zebra mussels.

eDNA can be isolated from the water by collecting a volume of water and filtering it and concentrating the DNA onto small filters. The DNA can then be extracted off the filters using typical methods in the laboratory and subjected to DNA-based techniques such as qPCR. For quagga and zebra mussels, several qPCR tests have been designed and published in the recent literature [1–5], and it has been recognized that eDNA offers a very sensitive and reliable way to monitor for these invasive species [4–7], outperforming manual searches in cases where methods have been compared [2]. CLWA funded us to collect water samples, isolate eDNA, and use qPCR to test for the presence of quagga mussels in Crystal Lake. This report documents our findings.

3095 Pender Ct Adrian, MI 49221 cblankes@umich.edu www.swimmersitchsolutions.com

Methods

Water sample collection

Water samples were collected in September 2022 at 10 sites on Crystal Lake (the same ten sites as are used for snail surveys). A preservative recognized as effective in the eDNA literature was added to prevent DNA degradation. Late summer and early fall are good times to use eDNA to sample for quagga and zebra mussels, as at least one study showed higher levels of mussel eDNA in fall than in the spring [6]. This is thought to be because the mussels are active and growing through the summer months, as well as spawning and releasing larvae in mid- to late summer, many of which die and their DNA is released as eDNA.

Due to a series of problems getting reliable results in the laboratory, it was eventually concluded that the 2022 samples were not good and new samples had to be retaken. A second set of samples was taken in August 2023, and this time the preservative was not used. Because other laboratory issues had been resolved, we were able to obtain the results quickly and data and the conclusions in this report are based on those August 2023 samples.

Procedures

Water samples were kept on ice while in the field and transport to the lab and refrigerated on arrival. They were filtered within 48 hours, with each water collection divided into 3 subsamples of 0.5 L and each filtered onto a separate filter. Subsampling is strongly recommended for eDNA samples to increase confidence in results. A negative control of distilled water was also filtered for quality assurance purposes.

eDNA was then extracted from each filter, for a total of 30 DNA extractions. Species-specific qPCR tests (assays) from the literature were used to detect whether there is any quagga mussel or zebra mussel DNA. These tests consisted of the following:

Assay name	Target species	Target gene	Literature source
DRE16S	Both	16S ribosome	[1]
DREQM	Quagga mussels	Cytochrome oxidase I	[1]
ZEBCYT	Zebra mussels	Cytochrome b	[3]

Each subsample was scored as positive or negative separately for each of the three assays. Since zebra mussels are already numerous and widespread in Crystal Lake, it was expected that most or all subsamples would be positive for zebra mussel eDNA. This also means that it was expected that most or all samples would be positive for the DRE16S assay, which detects both species. The results are summarized in Table 1 on the next page.

Table 1. Results of qPCR assays on 30 subsamples from 10 collection sites on Crystal Lake.

Site	Subsample	DRE16S	QM	ZM	Greater eDNA amount*
River outlet	A	+	-	+	Zebra
	B	+	-	+	Zebra
	C	+	+	+	Equal
Onkeonwe Road	A	+	+	+	Quagga
	B	+	+	+	Quagga
	C	+	+	+	Quagga
CBCA	A	+	+	+	Quagga
	B	+	+	+	Quagga
	C	+	+	+	Equal
CSA	A	+	+	+	Zebra
	B	+	+	+	Zebra
	C	+	+	+	Zebra
Marquette Court	A	+	+	+	Zebra
	B	+	+	+	Zebra
	C	+	+	+	Zebra
Yacht Club	A	+	+	+	Equal
	B	+	+	+	Equal
	C	+	+	+	Equal
M6 Hotspot	A	+	+	+	Equal
	B	+	+	+	Equal
	C	+	+	+	Equal
Nichols Road	A	+	+	+	Quagga
	B	+	+	+	Equal
	C	+	+	+	Zebra
Orchard Hill	A	+	+	+	Zebra
	B	+	+	+	Zebra
	C	+	+	+	Zebra
Beulah	A	+	+	+	Equal
	B	+	+	+	Zebra
	C	+	+	+	Zebra
Totals		**30/30**	**28/30**	**30/30**	**6 Quagga** / **9 Equal** / **15 Zebra**
Negative control		-	-	-	
Quagga positive control		+	+	-	
Zebra positive control		+	-	+	

*If one test had a signal 2X or more that of the other. If less than 2X, the comparison was scored as approximately equal.

Summary and Implications

The high frequency of subsamples positive for quagga mussel eDNA (28/30) strongly suggests that Crystal Lake has an established population of quagga mussels. They are likely in deeper waters, as quagga mussels are typically more numerous than zebra mussels at depths more than 20 meters in the Great Lakes [8]. It is very unlikely that the test results are false positives, as the qPCR assays performed as expected and remained specific to their target species when checked with negative and positive controls (bottom of Table 1).

That all 30 subsamples were positive for the zebra mussel assay and the assay that detects both species is not surprising since the zebra mussel population is well established and easily observed on rocks and other hard surfaces throughout Crystal Lake. These results also lend support that all lab procedures were working well. Finally, comparing the relative amount of eDNA detected in the two assays is a somewhat crude measure, but it is suggestive that quagga mussels may not be as abundant as zebra mussels (or at least quagga eDNA was not as abundant in near shore water at the time of sampling).

Literature Cited

1. Gingera, T.; Bajno, R.; Docker, M.; Reist, J. Environmental DNA as a Detection Tool for Zebra Mussels Dreissena Polymorpha (Pallas, 1771) at the Forefront of an Invasion Event in Lake Winnipeg, Manitoba, Canada. *Manag. Biol. Invasions* **2017**, *8*, 287–300, doi:10.3391/mbi.2017.8.3.03.
2. De Ventura, L.; Kopp, K.; Seppälä, K.; Jokela, J. Tracing the Quagga Mussel Invasion along the Rhine River System Using EDNA Markers: Early Detection and Surveillance of Invasive Zebra and Quagga Mussels. *Manag. Biol. Invasions* **2017**, *8*, 101–112, doi:10.3391/mbi.2017.8.1.10.
3. Sepulveda, A.J.; Amberg, J.J.; Hanson, E. Using Environmental DNA to Extend the Window of Early Detection for Dreissenid Mussels. *Manag. Biol. Invasions* **2019**, *10*, 342–358.
4. Feist, S.M.; Lance, R.F. Advanced Molecular-Based Surveillance of Quagga and Zebra Mussels: A Review of Environmental DNA/RNA (EDNA/ERNA) Studies and Considerations for Future Directions. *NeoBiota* **2021**, *66*, 117–159, doi:10.3897/neobiota.66.60751.
5. Amberg, J.J.; Merkes, C.M.; Stott, W.; Rees, C.B.; Erickson, R.A. Environmental DNA as a Tool to Help Inform Zebra Mussel, Dreissena Polymorpha, Management in Inland Lakes. *Manag. Biol. Invasions* **2019**, *10*, 96–110.
6. Peñarrubia, L.; Alcaraz, C.; Vaate, A. bij de; Sanz, N.; Pla, C.; Vidal, O.; Viñas, J. Validated Methodology for Quantifying Infestation Levels of Dreissenid Mussels in Environmental DNA (EDNA) Samples. *Sci. Rep.* **2016**, *6*, 39067, doi:10.1038/srep39067.
7. Blackman, R.; Benucci, M.; Donnelly, R.; Hänfling, B.; Harper, L.; Sellers, G.; Handley, L. Simple, Sensitive and Species-Specific Assays for Detecting Quagga and Zebra Mussels (Dreissena Rostriformis Bugensis and D. Polymorpha) Using Environmental DNA. *Manag. Biol. Invasions* **2020**, *11*, 218–236, doi:10.3391/mbi.2020.11.2.04.
8. Mills, E.L.; Dermott, R.M.; Roseman, E.F.; Dustin, D.; Mellina, E.; Conn, D.B.; Spidle, A.P. Colonization, Ecology, and Population Structure of the "Quagga'' Mussel (Bivalvia: Dreissenidae) in the Lower Great Lakes. *Can. J. Fish. Aquat. Sci.* **1993**, *50*, 2305–2314, doi:10.1139/f93-255.