Assessment of the Efficacy and Environmental Impact of Zequanox® for Zebra Mussel Control Programs in Lakes and Reservoirs

A Case Study of Deep Quarry Lake
West Branch Forest Preserve, DuPage County, Illinois

Study Completed
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1.0 Acknowledgements

Marrone Bio Innovations, Inc. would like to thank the efforts of Professor Gregory Whitledge and his graduate student assistants from Southern Illinois University, and the staff of the Forest Preserve District of DuPage County for their dedication and hard work that went into making this project happen. We would also like to thank Kevin Irons and the Illinois Department of Natural Resources for their efforts in getting this study off the ground and assisting the group through the regulatory process.

2.0 Executive Summary

Zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*), bivalves native to Eastern Europe, are invasive species that were first discovered in North America in the late 1980s in the Great Lakes. Since then, these destructive invaders have spread throughout North America and have caused significant ecological and economic damage to areas they have infested. Because of their ability to reproduce quickly and in large numbers, these filter feeders can rapidly take over water systems they invade, outcompeting native species for food and space. The mussels settle in dense colonies on virtually all hard substrates. They have even been found colonizing indigenous native bivalve species (and other organisms), preventing them from being able to feed, and ultimately putting many native bivalve species at risk for extinction. The mussels' ability to filter large quantities of water in a single day can completely obliterate food sources for important fish species, ultimately resulting in population crashes. Invasive mussels also clog boat engine intake systems, causing the motors to overheat, and coat boat hulls, resulting in costly damage to paints and coatings. In addition, their thin, sharp shells cause painful cuts and wounds, and when the shells of deceased mussels wash ashore, they can turn popular recreational beach areas into malodorous, deserted eyesores.

Currently, there are no commercially viable alternatives for treatment of invasive mussels in open water systems. Water resource managers are continuing to search for effective, environmentally compatible methods to control invasive mussels. As a part of this effort, the Illinois Department of Natural Resources awarded grant money from the U.S. Fish and Wildlife Service to Dr. Gregory Whitledge of Southern Illinois University (SIU) to conduct a study using Zequanox®, a naturally derived biopesticide effective on zebra and quagga mussels, on an infested lake in Illinois. Deep Quarry Lake, in the Forest Preserve District of DuPage County (District), was identified as an ideal study site and Marrone Bio Innovations, Inc. (MBI), the maker of Zequanox, was contacted to collaborate on the project.

Project Objectives:

- Demonstrate Zequanox product application effectiveness at controlling invasive mussel populations in open water and natural systems.
- Document Zequanox application performance on invasive mussel populations in Deep Quarry Lake.
- Develop local and state partnerships to enhance Zequanox product development for open water applications.

Results Summary:

- Applying Zequanox in barriers systems is an effective method of product application for zebra mussel control (Note: quagga mussels are not present in Deep Quarry Lake).
- Treated sites had an average mussel mortality of 97.1%, compared to 11.2% mortality in control sites.
- No juvenile or adult fish mortality was observed within treated sites 24 hours after product application.
- Water quality monitoring results indicated no lasting effects on water quality.

3.0 Introduction

Zequanox, which is made from a strain of the naturally occurring bacteria *Pseudomonas fluorescens* (*P. fluorescens* strain CL145A), is a biopesticide effective at controlling invasive zebra and quagga mussels. The product has been commercially developed by MBI. *Pseudomonas fluorescens* is a common soil bacterial species found worldwide and is known to protect the roots of plants from disease. Like other successful biopesticides that are used globally to control pests such as black fly and mosquito larvae, Zequanox is an environmentally compatible control option for invasive mussels. When applied, the active ingredient of Zequanox (i.e., dead *P. fluorescens* CL145A cells), is non-toxic to humans and is highly selective, affecting only zebra and quagga mussels.

This report presents the design, results, and conclusions of a study implemented in partnership with the Illinois Department of Natural Resources, SIU, the District, and PLM Lake and Land Management (PLM), collectively referred to as the study team. This study utilized barrier systems for limited scale treatments in Deep Quarry Lake with Zequanox. Two independent analyses of mussel mortality were completed as part of this cooperative project. This report focuses on the methods used by MBI and includes summary information of the efforts of SIU. These analyses provide independent validation of results as part of the private-public partnership nature of this study.

MBI completed mortality assessments of collected zebra mussels using containment chambers and found the Zequanox applications to be highly effective: 97.1% mortality in treated sites compared to 11.2% mortality in control sites. Water quality monitoring before, during, and after product application indicated no lasting water quality impact associated with product application. No adult or juvenile fish mortality was observed inside the treatment barriers 24 hours after product application.

4.0 Roles and Responsibilities

- **Marrone Bio Innovations, Inc.**—project coordination, staff and equipment resources, study design, and data collection
- **Southern Illinois University**—project coordination and substrate mortality monitoring
- **Forest Preserve District of DuPage County**—project coordination, lake managers, staff and equipment resources
- **Illinois Department of Natural Resources**—project initiation, permitting/regulatory assistance, and grant funding for SIU activities
- **PLM Lake and Land Management**—professional pesticide application and project consultation

5.0 Methods

5.1 Study Site

Deep Quarry Lake is within the West Branch Forest Preserve near Bartlett, Illinois. The lake, which has a surface area of approximately 40 acres and a maximum depth of 45 feet, has no naturally occurring tributaries and only outflows during flood and high-water events. Water contact recreation is prohibited at Deep Quarry Lake, and it is a popular urban fishing location containing bass, sunfish, channel and flathead catfish, carp, bluegill, walleye, northern pike, and crappie. Zebra mussels were first discovered in Deep Quarry Lake in 2009 and have since developed a well-established population within the lake.

Three sets of paired, 24-square-meter (m^2) treatment and control sites were established within Deep Quarry Lake. One set was placed along the west shoreline (Figure 1, Sites T1 and C1) and two sets were placed along the east shore (Figure 1, Sites T2, C2, T3 and C3). The study team selected sites based on the presence of settled zebra mussels throughout the 24-m^2 site, as well as accessibility, lack of steep drop-offs, and relatively equivalent depth within the sites. Control sites were always placed as far away from treatment sites as possible given the bathymetric characteristics of the lake, to minimize effects of water current and wind, to prevent contamination of control sites by drift from treatment sites.

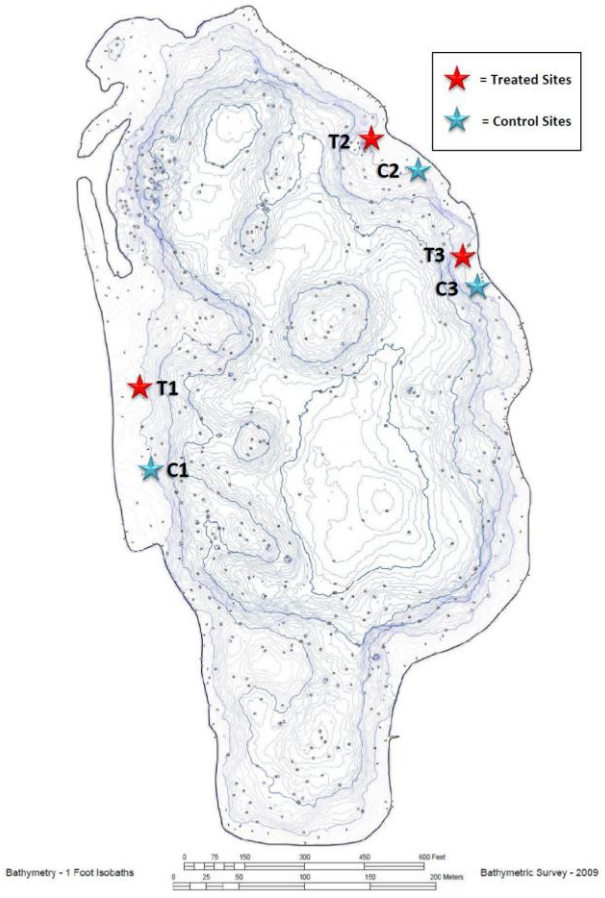

Figure 1 – Locations of Treatment and Control Sites in Deep Quarry Lake
(Image modified from Forest Preserve District of DuPage County map)

5.2 Barrier Systems and Product Application

Barrier systems were used during product application to maintain product concentration for treatment durations. This study used customized Type II turbidity curtains manufactured by Elastec/American Marine. The day before each treatment, the PVC barrier curtains were fully installed around each previously marked treatment and control site. Barriers were deployed at both treatment and control locations to demonstrate that the barriers themselves were not having an effect. The curtains were 8 m long and 3 m wide. Curtain walls were furrowed to the appropriate depth to prevent the curtain walls from bowing into the plot areas, and sandbags were placed along the perimeter to hold the furrowed curtain and anchor the walls to the lake bottom. In addition, the curtains were equipped with a skirt, approximately 0.5 m wide with a heavy chain anchor, to create an additional barrier along the lake bottom, to minimize any seepage of Zequanox outside of treated areas and to further anchor the curtains. Crews from the District and MBI, along with SIU personnel and students, installed each of the barriers without disturbing plot areas within the barriers.

Site T1 was treated July 18, 2012 and sites T2 and T3 were treated July 19, 2012. On the treatment day, MBI and PLM personnel mixed Zequanox to a 10% solution. The target treatment zone was the bottom 0.75 m of the treated corral(s). Additional product was mixed to account for product loss due to diffusion to the upper layer and possible seepage through curtain seams. Product mixing occurred at the District's pesticide mixing facility and was then transferred to a holding tank onboard the PLM application boat before being transported to Deep Quarry Lake. PLM staff injected the Zequanox solution into the bottom layer of the treated sites by application wand to reach a target treatment concentration of 150 milligrams active ingredient per liter (mg a.i./L), 300 mg/L as total product weight (Figure 2).

Figure 2 - Zequanox Application and Treated Site after Application

Because Zequanox concentration has a linear relationship with turbidity, turbidity measurements can be used to accurately estimate actual concentration. MBI staff determined target turbidity by filling three beakers with 500 milliliters of lake water and dispensing the proper amount of stock solution to reach a concentration of 150 mg a.i./L (300 mg/L total). The average of the turbidity readings of the three beakers was recorded as the target turbidity for the desired concentration.

MBI monitored turbidity over a 6-hour treatment period. When concentration within the treated barriers fell below the determined target turbidity during the 6-hour treatment period, additional stock solution was applied until the end of the treatment period, or until all mixed product was applied. Treated water was contained within the curtains for an additional 18 hours. The study team removed the curtains the morning after treatment in a step-wise fashion to allow treated water to slowly diffuse into untreated water.

5.3 Water Quality Monitoring

MBI staff monitored water quality parameters during the study period. Water samples were collected from each site prior to application and then 1, 3, 7, and 14 days post application for analysis of ammonia, total nitrogen and total phosphorus by an independent lab. At site T1 and C1, biochemical oxygen demand and chlorophyll *a* were also monitored. Chlorophyll *a* was monitored at 2 days post application rather than 3 days due to lab availability for analysis. MBI staff used a multi-parameter water quality meter to monitor pH, temperature, turbidity, conductivity, and dissolved oxygen (DO) on the schedule described above. The water quality meter was also used during treatment to regularly monitor pH, temperature, turbidity, conductivity, and DO in each treated and control site. Table 1 shows a summary of water quality parameters measured in each corral. All water samples and water quality meter readings were consistently from the same marked location within each site, 0.5 m from the lake bottom.

Table 1. Water Quality Parameter Monitoring Summary by Site

Site	T1	C1	T2	C2	T3	C3
BOD	X	X				
Chlorophyll *a*	X	X				
Total N	X	X	X	X	X	X
Ammonia	X	X	X	X	X	X
Total P	X	X	X	X	X	X
Temperature	X	X	X	X	X	X
Turbidity	X	X	X	X	X	X
Conductivity	X	X	X	X	X	X
DO	X	X	X	X	X	X
pH	X	X	X	X	X	X

5.4 Mussel Mortality Monitoring

The study team collected zebra mussels by hand from rocks and other hard substrate within Deep Quarry Lake two weeks prior to treatment and placed them into mesh bags, which were then stored in the lake. Collected mussels were sorted and selected for healthy, responsive mussels. Fifty selected mussels were placed into each of 18 mussel containment chambers (Figure 3) and 3 chambers were placed into each treated and control site. Zebra mussel mortality in containment chambers within each site was monitored for up to 14 days post treatment. Mussels were classified as dead if they were open and non-responsive to external stimuli.

Figure 3 - Mussel Containment Chambers

The day following treatment and again, 1 week post treatment, SIU conducted mortality monitoring by randomly selecting three 1 m^2 sample plots within each treated and control site. Samples of the benthic substrate within these plots were collected and analyzed for dead and live mussels. Mortality monitoring of mussels in the benthic environment provided complementary data for mussels in the natural environment that were not handled or otherwise disturbed by the collection process used in MBI methodology.

6.0 Results

Zequanox diffused quickly into the upper layer in all treated sites. After the first turbidity reading following initial application, the concentration was below target concentrations and the remaining stock solution was dispensed into the corral. Final concentrations were calculated based on measured volume of water in each treated corral and the known volume of Zequanox stock solution. Concentrations were determined to be 124 mg a.i./L (248 mg/L total) in site T1, 115 mg a.i./L (230 mg/L total) in site T2, and 93 mg a.i./L (186 mg/L total) in site T3. As expected in natural settings, turbidity readings with the water quality meter were not uniform across individual treated sites, likely indicating areas of localized high and low concentrations.

Water quality monitoring conducted by MBI staff during treatment showed no effect on DO, pH, conductivity, or temperature when comparing treated and control sites. Turbidity in treated sites increased after the initial application and after the second application (remaining stock solution), and then began to taper off during the treatment period (Figure 4). The treatment period included the first 6 hours (360 minutes) following the initial application. This period was previously designated as the time period during which additional mixed Zequanox would be added to maintain the target concentration (until all mixed solution was used). Maximum observed turbidity was 102 Nephelometric Turbidity Units (NTU). Turbidity dropped off significantly the day after treatment in treated sites, with an average turbidity of 9 NTU 24 hours after initial application, compared to 1.2 NTU in control sites. A temporary drop in DO was observed 24 hours after initial application within the treatment site barriers; however, all sites returned to background DO levels quickly. Average water temperature during treatment was 28.7°C.

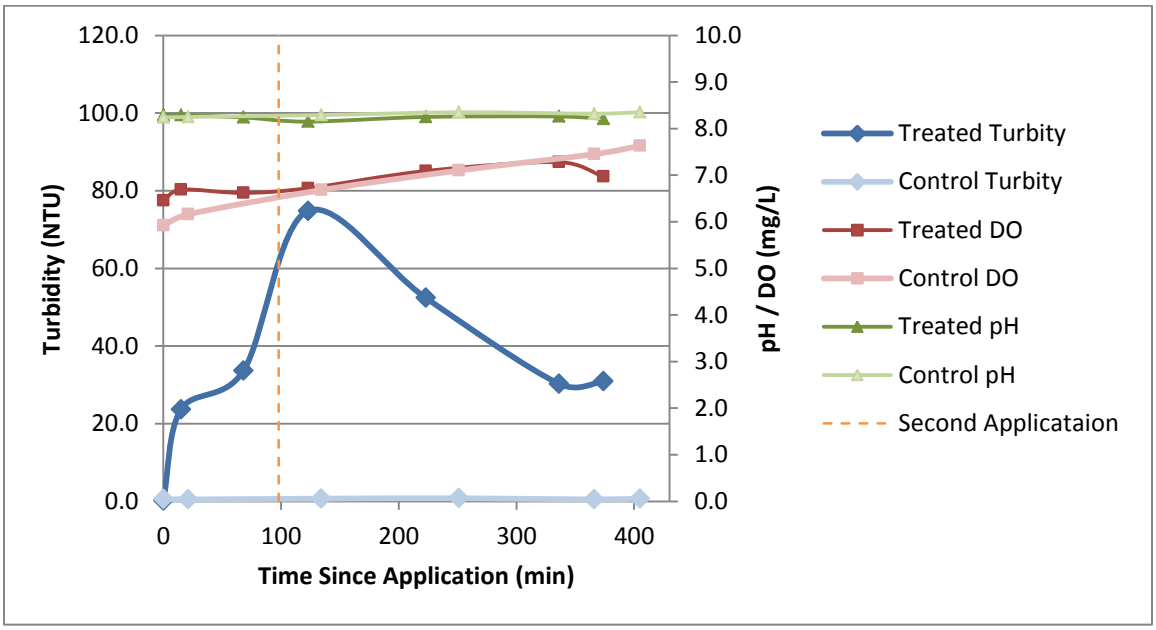

Figure 4 – Turbidity, DO, and pH Readings during Treatment at Sites T2 and C2

Results from water samples analyzed for ammonia, total nitrogen, total phosphorus, biochemical oxygen demand, and chlorophyll *a* showed no impact for all parameters (Table 2).

Table 2. Results from Water Samples Collected at Deep Quarry Lake

Parameter	Time Elapsed (days)	Site					
		T1	C1	T2	C2	T3	C3
BOD (mg/L)	0	<2	<2	NT	NT	NT	NT
	1	<2	<2	NT	NT	NT	NT
	3	<2	<2	NT	NT	NT	NT
	7	<2	<2	NT	NT	NT	NT
	14	<2	2.5	NT	NT	NT	NT
Chlorophyll *a* (mg/L)	0	ND	ND	NT	NT	NT	NT
	1	ND	ND	NT	NT	NT	NT
	2	ND	ND	NT	NT	NT	NT
	7	ND	ND	NT	NT	NT	NT
	14	ND	ND	NT	NT	NT	NT
Total Phosphorus (mg/L)	0	0.032	0.042	0.025	ND	0.025	0.028
	1	0.032	0.03	0.028	0.028	0.021	0.028
	3	0.028	0.028	0.028	ND	0.028	0.023
	7	0.032	0.032	0.032	0.028	0.021	0.042
	14	0.023	0.021	0.058	0.093	0.079	0.037
Total Nitrogen (mg/L)	0	ND	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND	ND
	14	ND	ND	ND	ND	ND	ND
Ammonia (mg/L)	0	ND	ND	ND	ND	ND	ND
	1	ND	ND	ND	ND	ND	ND
	3	ND	ND	ND	ND	ND	ND
	7	ND	ND	ND	ND	ND	ND
	14	ND	ND	ND	ND	ND	ND

Note: ND = not detected, NT = not tested

Mortality monitoring conducted by MBI staff showed that Zequanox treatment was highly effective against invasive zebra mussels in all three treated sites with an average of 97.1% mortality across all three sites and a control mortality of 11.2% (Figure 5). Table 3 presents results for individual sites. As a result of the partnerships developed in this project, private and academic institutions assessed Zequanox treatment efficacy utilizing different methods. Figure 6 shows the results from SIU assessments, which support the results found by MBI with average treated site mortality of greater than 90% compared to a control site mortality of less than 10% one week after treatment.

Visually, significant mussel mortality was observed by the study team (as gaping shells) in treated sites the day following treatment. Additionally, 24 hours after application, fish were found swimming in the treated barriers with no observed mortalities.

Table 3. Summary of Final Zebra Mussel Mortality Monitoring Results

Site	Check Day	Mean Mortality (%)	Standard Deviation (+/- %)
Treated 1 (T1)	14	96.7	3
Control 1 (C1)	14	7.3	2.3
Treated 2 (T2)	13	98.7	1.1
Control 2 (C2)	13	13.4	1.2
Treated 3 (T3)	13	96	5.3
Control 3 (C3)	13	12.8	6.3

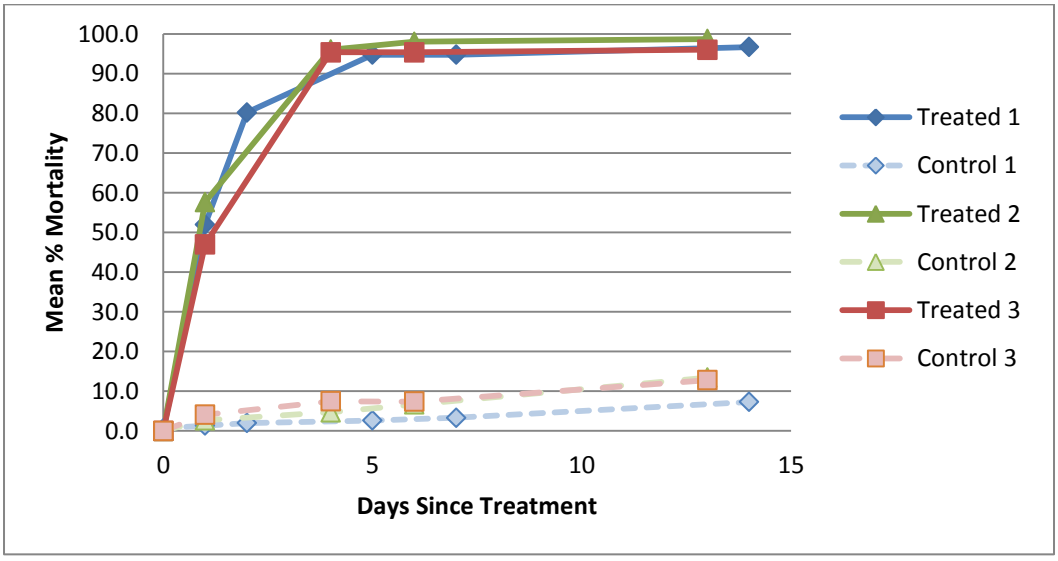

Figure 5 - Mussel Mortality from MBI Assessments at Deep Quarry Lake

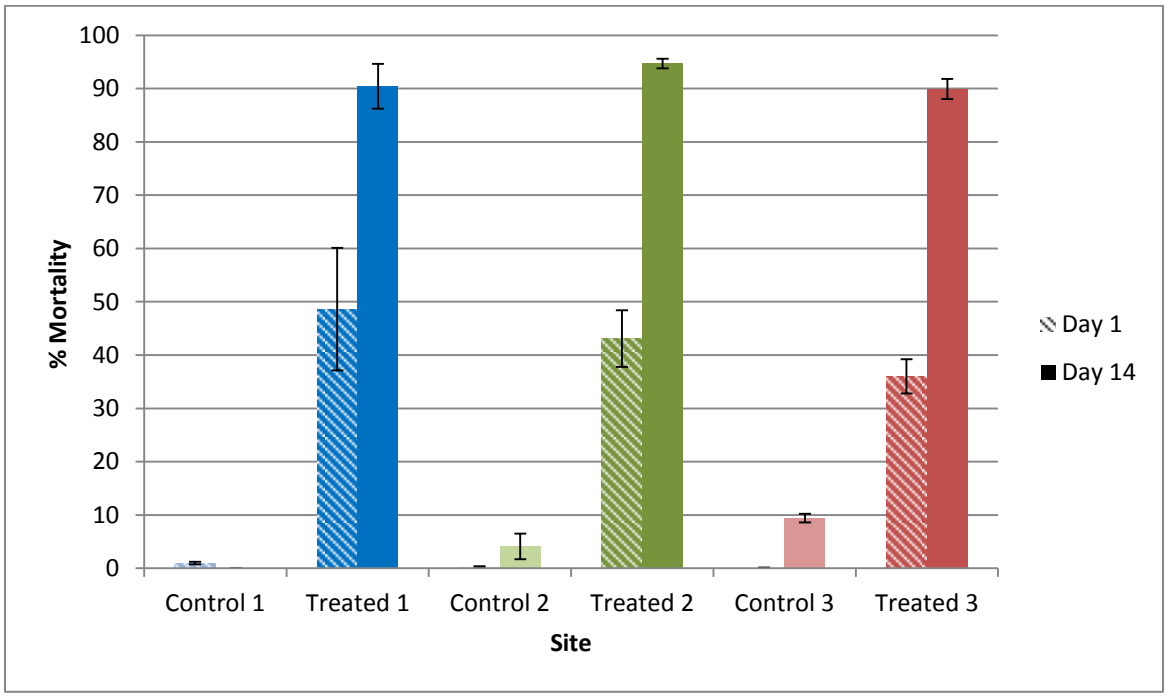

Figure 6 - Mussel Mortality from SIU Assessments at Deep Quarry Lake

7.0 Conclusions

When utilized within barrier systems, Zequanox is highly effective in controlling invasive zebra mussels, with zebra mussel mortality in treated sites reaching 97.1% within 14 days, compared to only 11.2% in control sites. Analysis of the benthic substrate by SIU supports these results. Analysis of water quality parameters showed no lasting negative effects on the aquatic environment. No juvenile or adult fish mortality was observed in treated locations 24 hours after product application. Results of this study suggest that Zequanox can be an effective tool in the control of invasive mussels in natural systems without causing significant adverse effects on water quality or the environment.

The success of this study was built on a strong public-private partnership. Cooperative planning involving all members of the study team resulted in a robust project addressing the needs and questions of multiple players while reducing costs through resource sharing. The resulting independent validation of results adds further strength to the conclusions of this study.