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Gregory Whitledge
Southern Illinois University Carbondale, gwhit@siu.edu

Megan M. Weber

Jessi DeMartini

John Oldenburg

Dave Roberts

See next page for additional authors

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Research Article

An evaluation Zequanox® efficacy and application strategies for targeted control of zebra mussels in shallow-water habitats in lakes

Gregory W. Whitledge[1]*, Megan M. Weber[2], Jessi DeMartini[3], John Oldenburg[3], Dave Roberts[2], Carolyn Link[2], Sarahann M. Rackl[2], Neil P. Rude[1], Andrew J. Yung[1], Lindsey R. Bock[1] and Devon C. Oliver[1]

[1]*Center for Fisheries, Aquaculture, and Aquatic Sciences, Southern Illinois University, Carbondale, Illinois 62901-6511, USA*
[2]*Marrone Bio Innovations, Inc., 2121 2nd St Suite B-107, Davis, CA 95618, USA*
[3]*Forest Preserve District of DuPage County, Wheaton, IL 60189, USA*

**Corresponding author*
E-mail: gwhit@siu.edu

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Abstract

An evaluation of Zequanox® (a naturally derived biopesticide that is non-toxic to humans and other aquatic life and selectively kills dreissenid mussels) for controlling zebra mussel infestations in shallow-water habitats in lakes was conducted at Deep Quarry Lake in DuPage County, Illinois during summer 2012 and 2013. During the 2012 trial, three sets of paired 24-m² treatment and control sites were established within the lake, while a single 324-m² treatment site was established for the 2013 trial. Zequanox was applied to treatment plots, contained using PVC barrier curtains, and barriers were removed during the morning following application. Zebra mussel mortality and size distributions on natural substrates were assessed one day and one week post-treatment for 2012 trials and one day and two weeks post-treatment for 2013 trials; percent mortality of zebra mussels in mesh containers in treatment and control sites was also monitored up to 14 days and 48 days post-treatment in 2012 and 2013, respectively. Several water quality parameters were measured in control and treatment plots before and during application and up to 14 d post-treatment. Mean percent mortality for adult zebra mussels on natural and artificial substrates in treatment locations 7–48 d following Zequanox application ranged from 92–98% during both years, while mortality was consistently ≤10% in control locations. Mean percent mortality ranged from 15–76% in locations > 5 m from and in water shallower than Zequanox application points (<0.6 m depth) during the 2013 trial likely due to limited product dispersal into these areas. There was no significant difference in the size distribution of live and dead zebra mussels in treatment plots. Mean veliger mortality was 94.4% 20-h after the start of the 6-h Zequanox treatment period in the treatment area compared to 15.1% in untreated locations during the 2013 trial. Temporary but substantial reductions in dissolved oxygen were observed in treatment locations during the morning following Zequanox treatment in both 2012 and 2013 trials, likely due to the presence of the barriers that prevented well-oxygenated water from circulating into treatment zones from adjacent areas in the lake. Dissolved oxygen concentrations quickly rebounded to levels consistent with control sites upon removal of barriers. No effects of Zequanox treatment on ammonia, total nitrogen, total phosphorus, biochemical oxygen demand, chlorophyll *a*, pH, or conductivity were observed. Results suggest that Zequanox has potential as a tool for controlling zebra mussels in shallow-water habitats in lakes without significant long-term effects on water quality.

Key words: *Dreissena polymorpha,* biopesticide, control, field trial, mortality, Deep Quarry Lake, Illinois

Introduction

Zebra and quagga mussels (*Dreissena polymorpha* Pallas, 1771 and *Dreissena bugensis* Andrusov, 1897), bivalves native to Eastern Europe, are invasive species that were first discovered in North America in the late 1980s in the Great Lakes (O'Neill and MacNeill 1989). Zebra mussels have since spread and are now found in 30 states in the U.S. and two Canadian provinces (USGS 2013). Zebra and quagga mussels have caused significant economic damage to many areas they have infested (Connelly et al. 2007) and can substantially alter energy flow, community structure, trophic interactions, and population dynamics of native species across multiple trophic levels in invaded ecosystems (Marsden and Chotkowski 2001; Raikow et al. 2004; Pothoven and Madenjian 2008; Sousa et al. 2014). Unfortunately, there are currently no commercially viable, environmentally safe methods available for control of dreissenid mussels in open water systems such as lakes and

rivers. Concentrations of currently approved chemical molluscicides required to achieve zebra and quagga mussel control in these systems are generally sufficient to cause mortality in most other aquatic organisms, including important fish and native bivalve species (USEPA 2008). While application of 131,000 kg of potassium chloride eliminated zebra mussels from Millbrook Quarry, Virginia in 2006, no native species of bivalves were present in the lake, and the target potassium treatment concentration used (100 ppm) would likely be lethal to non-target bivalves in lakes or rivers where they occur (VDGIF 2005; Sousa et al. 2014).

Zequanox® is a biopesticide manufactured by Marrone Bio Innovations, Inc. (MBI) for control of zebra and quagga mussels. Zequanox contains a killed strain *(Pf*-CL145A) of *Pseudomonas fluorescens* Migula, 1895, a common species of bacteria that occurs naturally in water and soil, and selectively kills dreissenid mussels (Molloy et al. 2013c). The commercial formulation of Zequanox at the time of testing was a spray-dried powder (SDP) consisting of 50% active ingredient (killed *Pf*-CL145A cells) and 50% inert ingredients (MBI 2012a). Tests of Zequanox in the laboratory and at power plants and dams to date have consistently indicated 70–100% mortality of zebra and quagga mussels exposed to this product, depending in part on water temperature (MBI 2012b; Molloy et al. 2013a, c). Zequanox's mode of action in killing dreissenids is intoxication (not infection) due to the presence of a natural metabolic product associated with the bacterium's cell wall; death is associated with selective destruction of the mussel's digestive tract epithelium (Molloy et al. 2013b). The active ingredient in Zequanox is non-toxic to humans (MBI 2014). The commercial product is completely biodegradable and toxicity of the naturally occurring metabolite to dreissenids significantly degrades within 24 h of application to water (MBI 2014; Molloy et al. 2013a, b). Previous studies have indicated that Zequanox treatments pose minimal to no risk to non-target species, including many fish species, numerous species of native North American unionid mussels, other aquatic invertebrates (*Hyalella* and *Daphnia*), and aquatic plants (MBI 2014; Molloy et al. 2013c; Pletta 2013). While investigations of the efficacy of Zequanox as a selective dreissenid control agent in the laboratory and in enclosed systems, such as at power plants, have suggested its potential applicability to natural systems, this product had not yet been tested in open waters such as lakes, rivers, or constructed waterways (harbors, canals) prior to the initiation of this study. Additional evaluation of Zequanox under field conditions would be valuable to more fully assess its applicability as a mussel control agent in open water settings.

The objective of this study was to conduct field trials to assess the use of Zequanox SDP for controlling zebra mussels in shallow-water habitats in lakes for the purpose of potentially limiting the nuisance and ecological impacts of these introduced species.

Study area and methods

Study area

Deep Quarry Lake is located within the West Branch Forest Preserve near Bartlett, Illinois. The lake has a surface area of 16.187 hectares, a maximum depth of 13.72 m, no tributaries, and only outflows to the West Branch DuPage River during floods. Zebra mussels were first discovered in Deep Quarry Lake in 2009. The lake is popular for recreational angling; however, water contact recreation is prohibited at Deep Quarry Lake to limit the potential for zebra mussels being transported to other nearby lakes. No federally threatened or endangered species (or species proposed or candidates for listing) and no state threatened or endangered fishes or aquatic invertebrates are present in Deep Quarry Lake or the adjacent West Branch of the DuPage River.

2012 Field Trial

Three sets of paired, 24-m² 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). Treatment and control sites were selected based on the presence of settled zebra mussels throughout the area, accessibility, lack of steep drop-offs, and relatively consistent depth and limited macrophyte coverage within and among sites. Control sites were placed at depths and in visually similar habitat as treatment sites, but sufficiently far from treatment sites to prevent contamination of control sites by drift of Zequanox SDP from treatment sites following barrier removal (see below).

Barrier curtains (8 m long × 3 m wide; customized Type II, PVC turbidity curtains manufactured by Elastec/American Marine) were used to maintain Zequanox SDP concentration in

treatment plots during a 6-hour treatment period (Figure 2). One day prior to product application, barrier curtains were installed around each previously-marked treatment and control site. Curtain walls were furrowed to the appropriate depth to prevent 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; the skirt served as an additional seal along the lake bottom to minimize potential seepage of Zequanox SDP outside of treated areas and to further anchor the curtains. Barriers were installed without disturbing substrates within enclosed treatment or control plots.

Site T1 was treated July 18, 2012 and sites T2 and T3 were treated July 19, 2012. On treatment days, Zequanox SDP was mixed to form a 10% solution. The target treatment zone was the bottom 0.75 m of the water column within the barrier (average depths of treatment sites were 1.2, 1.3, and 1.6 m for sites T1, T2, and T3 respectively). Additional product was mixed to account for up to 30% product loss due to diffusion to the upper layer and possible seepage through curtain seams. Product mixing occurred at the Forest Preserve District of DuPage County's pesticide mixing facility; product was then transported to Deep Quarry Lake in the holding tank onboard the

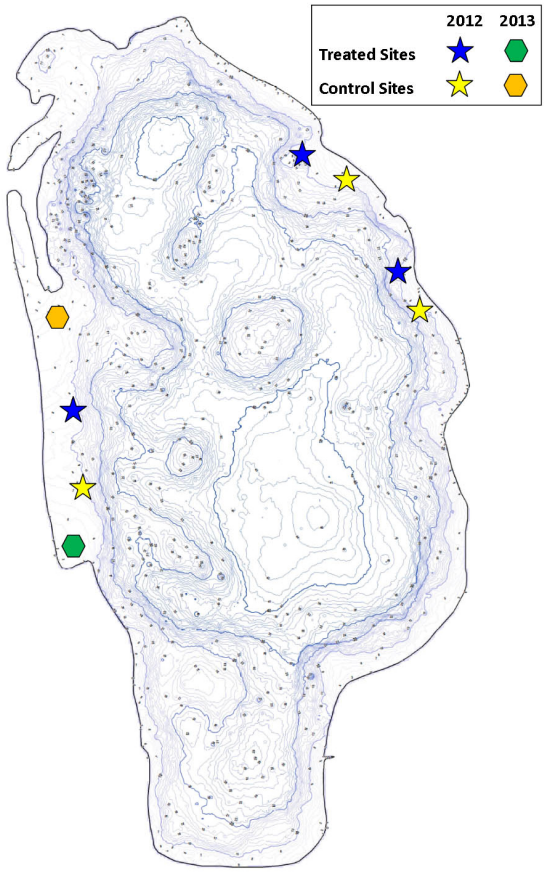

Figure 1. Map of Deep Quarry Lake showing locations of treatment and control sites during the 2012 and 2013 trials.

Figure 2. One of the PVC barrier curtains deployed in Deep Quarry Lake for the 2012 trial. Barriers were used to enclose treatment and control plots. Photograph by Dave Roberts.

application boat. The Zequanox SDP solution was injected into the bottom layer of the water column in treated sites by application wand to reach a target treatment concentration of 150 mg active ingredient per liter (mg a.i./L; 300 mg/L total product). Because Zequanox SDP concentration is linearly related to turbidity, turbidity measurements were used to estimate actual concentration. Target turbidity was determined by filling three beakers with 500 ml of lake water and dispensing the proper amount of stock solution to reach a concentration of 150 mg a.i./L. The average of the turbidity readings of the three beakers was recorded as the target turbidity for the desired concentration. Turbidity within treatment plots was monitored over a 6-hour treatment period. When initial turbidity readings indicated that the target concentration was not reached and that there had been significant product diffusion to the upper layer of water, the additional mixed product was applied to the treatment sites. Application of the additional mixed product occurred 1 h to 1.5 h after initial application. Barrier curtains were removed from treatment and control plots 18 h following the treatment period (the morning after treatment) and residual product was allowed to disperse from treatment plots. Barrier removal occurred the following morning because staff, equipment availability, and site access at the study site were limited in the evening hours after treatment.

Water samples were collected from each site using a Van Dorn sampler prior to application and then 1, 3, 7, and 14 days post-treatment for analysis of ammonia (method: SM-M4500NH3 F-Rev 18Ed, 1992), total nitrogen (sum of ammonia, total Kjeldahl N (method: SM-M4500Norg C and M4500NH3 C-Rev 18Ed, 1992), and nitrate/nitrite (method: MCAWW-353.2-Rev 2.0, 1993)) and total phosphorus (method: SM-M4500P BE-Rev 18Ed, 1992). At sites T1 and C1, biochemical oxygen demand (method: SM-M5210B-Rev 18Ed, 1992) and chlorophyll *a* (method: SM-A10200H-Rev 21st, 2005) were also monitored. Water samples were stored on ice and transported to an independent lab (Suburban Laboratories, Inc., Hillside, IL) for analyses. Chlorophyll *a* was monitored at 2 days post application rather than 3 days due to lab availability for analysis. A multi-parameter water quality meter was used to monitor pH, temperature, turbidity, conductivity, and dissolved oxygen during the application periods and after treatment on the schedule described above. All water samples and water quality measurements were consistently taken from the same marked location within each site, 0.5 m from the lake bottom.

Zebra mussels attached to benthic substrates and macrophytes were collected from each control and treatment plot on the day following treatment and at one week post-treatment to assess mortality rates and mussel size distributions. Mortality assessments for zebra mussels attached to benthic substrates were conducted exclusively by Southern Illinois University (SIU) personnel. Three, 1-m² sample plots were randomly selected from each control or treatment site on each sampling date and all observed zebra mussels were separated from substrates collected throughout each sample plot. To assess product efficacy for killing zebra mussels on complex, three-dimensional surfaces, submerged tree limbs (7.5–10 cm diameter) that had been colonized by zebra mussels were obtained from Deep Quarry Lake, cut into approximately 1-m sections using a hand saw, and one section placed on the lake bottom in the center of each control and treatment site one day prior to Zequanox SDP application. Dead mussels were identified as individuals having gaping shells and that did not respond to physical touch by closing and remaining closed. Counts of live and dead mussels were conducted for each sample plot at 1 day and 14 days after treatment and for wood substrate introduced into control and treatment sites 1 day following treatments only. Subsamples of both live and dead zebra mussels from each control and treatment site were measured to the nearest 0.1 mm along the longest axis of the shell.

In addition to the naturally settled mussels, Zequanox SDP efficacy was assessed using collected mussels contained in rigid plastic mesh chambers. Mortality assessments for contained mussels were conducted by MBI personnel with assistance from Forest Preserve District staff. Prior to product application, adult zebra mussels were collected from substrate in Deep Quarry Lake. The collected mussels were sorted and considered healthy by observed siphoning action and responsiveness to touch by closing of the shell. Fifty apparently healthy mussels were placed into each of 18 chambers and three chambers were placed on the lake bottom within each treatment and control site. Mortality in the chambers was monitored up to 14 days after application. Dead mussels were identified as described above. On each monitoring day, dead mussels were recorded and discarded while live mussels were returned to the chamber. Chambers were stored in Deep Quarry Lake within each of their

respective test plots during the 14 day monitoring period.

Differences in zebra mussel mortality (expressed as the percentage of dead mussels) between control and treatment plots at one day and one week post-treatment for naturally settled mussels and at 14 d post-treatment for mussels contained in chambers were assessed using Wilcoxon rank-sum tests. Wilcoxon rank-sum tests were also used to assess differences between zebra mussel mortality on benthic substrates and wood substrates in control and treatment plots at 24 h after Zequanox SDP application. Differences in length-frequency distributions for naturally settled zebra mussels sampled from control and treatment plots and between live and dead naturally settled zebra mussels sampled from treatment plots were evaluated with Kolmogorov-Smirnov tests.

2013 Field Trial

The 2013 field trial was designed to evaluate product efficacy and dispersal when applied to select locations within an enclosed area inclusive of shoreline much larger than the 24-m² plots used in the 2012 trial. Mortality of zebra mussel veligers resulting from Zequanox SDP treatment was monitored in addition to adult zebra mussel mortality. One day prior to treatment, a PVC barrier curtain (18 m long × 18 m wide) was installed to enclose a portion of the littoral zone (1.7 m maximum depth) on the west side of Deep Quarry Lake; the shoreline represented the fourth "side" of the barrier (Figure 3). The area contained within the barrier did not overlap with treatment or control plots used in the 2012 trial (sites T1 and C1 in Figure 1). On August 7, 2013, a one-time application of Zequanox SDP (mixed to form a 5% solution) was made to 6–7 locations in each of two parallel transects extending from near shore to the deep water edge of the barrier within the enclosed plot (Figure 3). Zequanox SDP was applied by injection approximately 0.3 m above the substrate with a target concentration of 150 mg a.i./L. The barrier remained in place for 18 h following the 6 h treatment period and was removed during the morning following treatment.

A multi-parameter water quality meter was used to monitor pH, temperature, turbidity, conductivity, and dissolved oxygen at the surface and 0.3 m from the bottom of the water column at application and non-application locations within the barrier and at control sites outside of the barrier at 2.5, 4.5, and 23.5 h after the start of the treatment period.

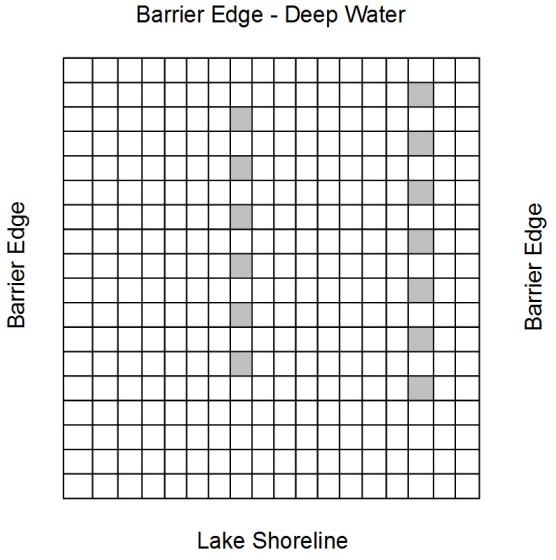

Figure 3. Diagram of treatment zones within the barrier during the 2013 field trial. Each box within the grid represents one square meter. Shaded boxes indicate locations of Zequanox® application points.

Zebra mussel veligers were sampled with 18-m horizontal tows using a 48-µm mesh plankton net. All veliger collections and mortality assessments were conducted exclusively by SIU personnel. Three plankton net tows were conducted perpendicular to shore near the north and south edges and through the center of the enclosed area after barrier installation one day prior to treatment. Three additional tows were conducted perpendicular to shore outside of the barrier on the day prior to treatment and again prior to barrier removal at both 3 and 20 h after the start of the Zequanox treatment period. Six tows (four evenly spaced and perpendicular to shore; two parallel to shore along the deepwater edge and through the center of the enclosed area) were also conducted within the enclosed area at both 3 and 20 h after the start of the Zequanox treatment period (prior to barrier removal). Veliger samples from each tow were rinsed into 250-ml collection bottles. Veliger samples were then stained with Fast Green dye and dead (stained mantle) and live (non-stained with mantle intact) veligers were counted under a dissecting microscope equipped with a cross-polarizing filter within 12 h of collection (Webb and Heasman 2006). Empty veliger shells (non-stained with no mantle intact) were also enumerated for each sample.

Settled zebra mussels attached to benthic substrates and macrophytes were collected by

SIU personnel from nine randomly selected 1-m² plots within the area that had been enclosed by the barrier to assess mortality rates at one day and again at two weeks post-treatment. Zebra mussels were also sampled from three randomly selected 1-m² plots outside of the treatment area (control samples) at one day and again at two weeks post-treatment. Live and dead mussels were distinguished as described for the 2012 trial. Mortality was also monitored for collected adult mussels contained in rigid plastic mesh chambers by MBI and Forest Preserve District personnel as described for the 2012 trial. Fifty healthy mussels were placed into each of 24 chambers. Twenty-one of the chambers were divided into five rows perpendicular to shore within the barrier and three were placed as controls 60 m north of the treated area to prevent any exposure from treated waters. The morning after treatment, all chambers were collected and stored in Deep Quarry Lake near the control site for the monitoring period. Mortality was monitored for up to 48 days after treatment.

Kruskal-Wallis one way ANOVAs were used to test for differences in veliger mortality (expressed as the percentage of dead individuals) and the percentage of empty shells among control and pre-treatment samples and samples collected 3 h and 20 h after the start of the Zequanox treatment period. Differences in veliger mortality rates for control samples over time were also assessed using a Kruskal-Wallis one way ANOVA. A Wilcoxon rank sum test was used to evaluate differences in veliger mortality between the 3h and 20 h sampling periods following the start of Zequanox application. Differences in settled zebra mussel mortality (expressed as the percentage of dead individuals) among control samples and at three sets of locations within the enclosed area (>5 m rom Zequanox SDP application points at water depths > 0.6 m, < 5 m from Zequanox SDP application points at water depths > 0.6 m, and areas shallower (<0.6 m deep) than application points) at one day and two weeks post-treatment were assessed using Kruskal-Wallis one way ANOVAs.

Results

2012 Field Trial

Zequanox SDP quickly spread throughout the water column within all treatment plots. After the first turbidity reading following initial application, turbidity measurements (used to estimated Zequanox SDP concentration) indicated that Zequanox SDP concentrations were below target concentrations. While target turbidity was determined to be 233 Nephelometric Turbidity Units (NTU), initial turbidity readings were 27.0 NTU, 33.6 NTU, and 8.5 NTU in sites T1, T2, and T3, respectively. After the addition of additional mixed product, turbidity within the treatment plots increased to 102 NTU in site T1, 74.8 NTU in site T2, and 58.4 NTU in site T3. Final concentrations (after application of the additional mixed product) were calculated based on measured volume of water in each treatment plot and the known volume of Zequanox stock solution. Concentrations were determined to be 124 mg a.i./L in site T1, 115 mg a.i./L in site T2, and 93 mg a.i./L in site T3.

Mortality for zebra mussels attached to benthic substrates and macrophytes in treatment plots averaged 42.6% (± 3.6% SE) one day following Zequanox SDP treatment and 91.7% (± 1.5% SE) at one week post-treatment and were significantly higher than zebra mussel mortality in control plots at one day (mean 0.41% ± 0.28% SE) and one week (mean 4.5% ± 2.7% SE) post-treatment, respectively ($p<0.05$; Figure 4). Mean percent mortality for zebra mussels on wood substrate placed in control plots (1.7% ± 1.6% SE) was significantly lower than that of mussels on wood substrate placed in treatment plots (37.9% ± 5.2% SE) at one day post-treatment ($p<0.05$). Mean percent zebra mussel mortality on benthic substrates in treatment plots was not significantly different from that of mussels on wood substrate in treatment plots one day following Zequanox SDP treatment ($p=0.78$); percent mortality also did not differ significantly between these two substrate types in control plots at one day post-treatment ($p=0.77$). Percent mortality in mussels contained in mesh chambers was also significantly higher in treatment plots (mean 97.1% ± 1.1% SE) than in control plots (mean 11.1% ± 1.5% SE) 14 days after treatment ($p<0.05$). Length frequency distributions of zebra mussels sampled from control and treatment plots were not significantly different ($p=0.37$; Figure 5). Length frequency distributions of live and dead zebra mussels sampled from treatment plots one day following Zequanox SDP treatment were also not significantly different ($p=0.97$; Figure 6).

Water quality monitoring conducted during the 6-h treatment period showed no effect on dissolved oxygen, pH, conductivity, or temperature in treatment plots compared to control plots.

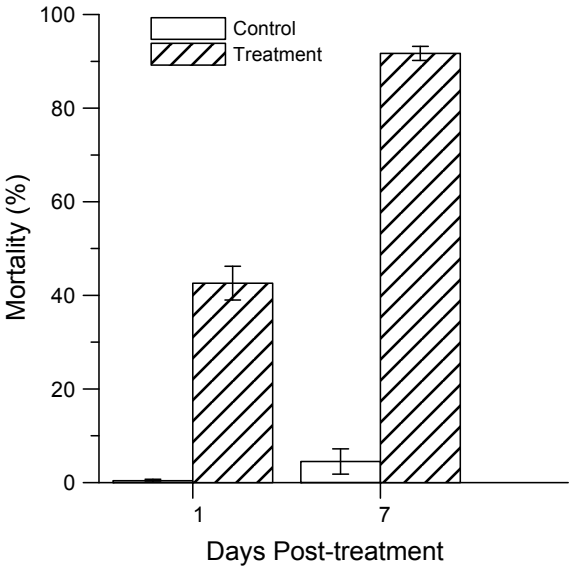

Figure 4. Mean percent mortality (±SE) for zebra mussels in control and treatment plots 1 d and 1 week following Zequanox® treatment in the 2102 trial.

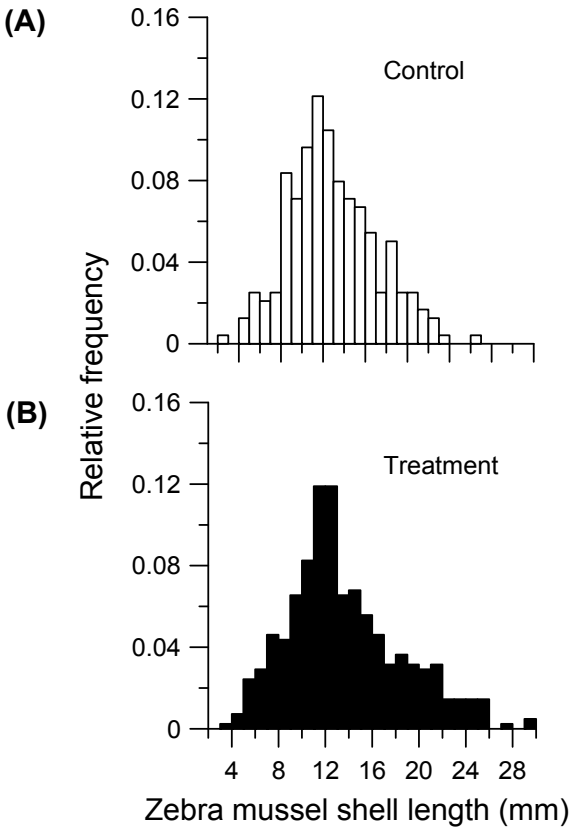

Figure 5. Length frequency histograms of zebra mussel shell lengths sampled from (A) control and (B) treatment plots during the 2012 trial. n=239 for controls, 412 for treatments.

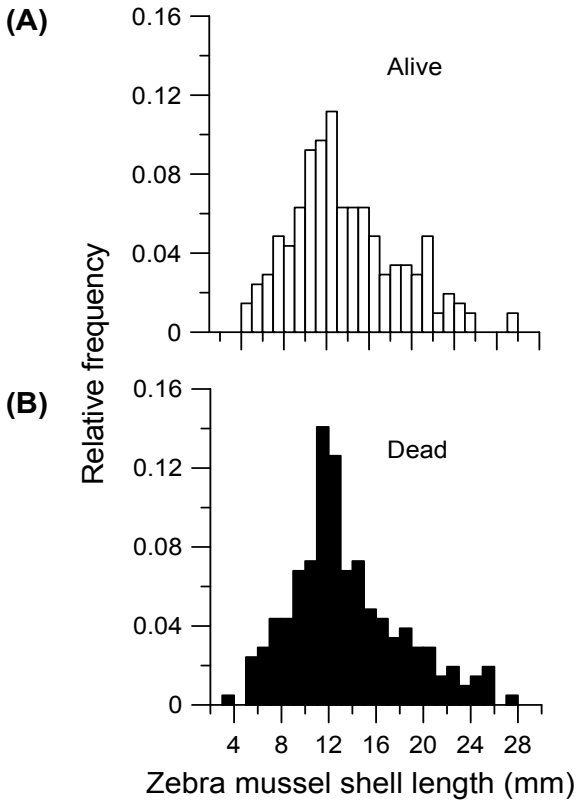

Figure 6. Length frequency histograms of shell lengths for (A) live and (B) dead zebra mussels sampled from treatment plots 1 d following Zequanox® treatment during the 2012 trial. n=206 alive and 206 dead individuals.

Turbidity in treated sites increased after the initial Zequanox SDP application and after the second application (remaining stock solution), then declined during the remainder of the time that barriers were in place for treatment plots. Maximum observed turbidity in treatment plots was 102 NTU. Turbidity dropped off significantly the day after treatment in treated sites, with an average turbidity of 9 NTU 24 h after the start of the treatment period compared to 1.2 NTU in control sites. A temporary drop in dissolved oxygen (to 2.5 mg/L) was observed 24 hours after the start of the treatment period within the treatment site barriers; however, all sites returned to background dissolved oxygen levels (6–8 mg/L) quickly when barriers were removed. Results from water samples analyzed for ammonia, total nitrogen, total phosphorus, biochemical oxygen demand, and chlorophyll *a* indicated no effect of Zequanox SDP treatment on any of these parameters (Table 1). Average water temperature during treatment was 28.7 °C.

Table 1. Water quality data from treatment (T1, T2, T3) and control (C1, C2, C3) plots prior to Zequanox application (day 0) and up to 14 days post-treatment for the 2012 trial. BOD = biochemical oxygen demand. ND = below detection limits (2 mg/L for BOD, 0.02 mg/L for total phosphorus, 0.1 mg/L for total nitrogen, and 0.1 mg/L for ammonia).

		Site					
Parameter	Time Elapsed (days)	T1	C1	T2	C2	T3	C3
BOD (mg/L)	0	ND	ND				
	1	ND	ND				
	3	ND	ND				
	7	ND	ND				
	14	ND	2.5				
Chlorophyll a	0	ND	ND				
	1	ND	ND				
	2	ND	ND				
	7	ND	ND				
	14	ND	ND				
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

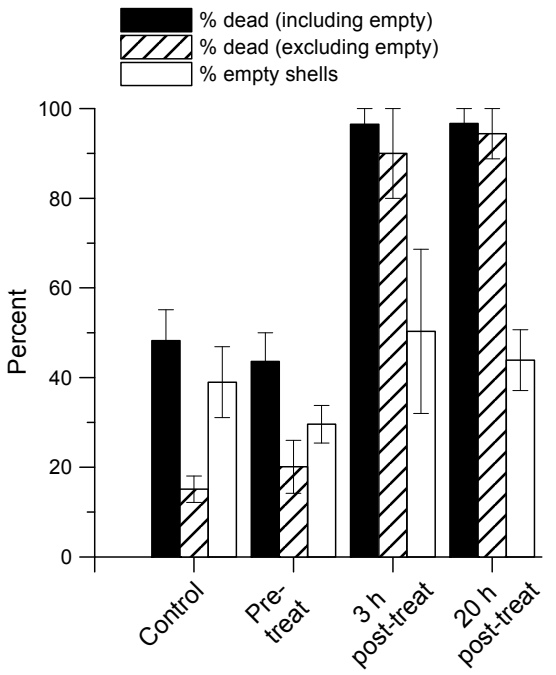

Figure 7. Mean percent mortality (± SE) for zebra mussel veligers and frequency of empty shells for control sites, pre-treatment sampling within the barrier, and at 3 h and 20 h following the start of Zequanox® treatment within the barrier during the 2013 trial.

2013 Field Trial

Zequanox SDP spread from the application points throughout the lower portion of the water column in the deeper portion (>0.6 m deep) of the area enclosed by the barrier creating a treatment layer in approximately the lower 0.3 m of the water column. Target turbidity for the 150 mg a.i./L treatment concentration was calculated at 166 NTU (target turbidity for maximum labeled rate was 224 NTU). Mean turbidity within the barrier immediately after completion of product application was 177.7 NTU (±11.3 NTU SE) near the substrate and 26.5 NTU (±0.6 NTU SE) at the water surface. Turbidity in areas shallower than application points was visually lower than in the rest of the treatment area. Mean turbidity was 4 NTU (±1.8 NTU SE) at locations outside of the area enclosed by the barrier. At 17.5 h following the 6 h Zequanox SDP treatment period (but before barrier removal), the product had distributed throughout the water column in the deeper portion (>0.6 m water depth) of the enclosed area (indicated by the similar turbidity readings from the surface and bottom samples), and mean turbidity had declined to 46.2 NTU (±4.6 NTU SE) for surface and bottom samples combined. Mean dissolved oxygen concentration

during the 6-h treatment period was 7.2 mg/L (±0.13 mg/L SE) inside the barrier and 8.5 mg/L (±0.2 mg/L SE) outside of the barrier. As in the 2012 trial, temporary reduction in dissolved oxygen (to 0.4 mg/L) was observed within the barrier 24 hours after initiation of the treatment period; however, dissolved oxygen levels rebounded to background levels within 2 hours of barrier removal. Conductivity averaged 673 µS (±6.8 µS SE) and pH averaged 7.8 (± 0.09 SE) during the 24 h monitoring period and were not substantially affected by Zequanox SDP application. Average water temperature during treatment was 26.2 °C. Percent mortality for zebra mussel veligers (excluding empty shells) averaged 15.1% (± 2.9% SE) in control samples (outside of the barrier), 20.1% (± 5.9% SE) in samples collected within the barrier on the day prior to Zequanox SDP treatment, and 90% (± 10% SE) and 94.4% (± 5.6% SE) for samples collected within the barrier at 3-h and at 20-h following the start of the Zequanox SDP treatment, respectively (Figure 7). Veliger mortality was significantly higher in samples collected following Zequanox SDP treatment compared to control and pre-treatment samples ($p<0.0001$). The percentage of empty veliger shells did not differ significantly among control and pre-treatment samples or samples collected subsequent to the start of Zequanox SDP treatment ($p=0.18$; Figure 7). Percent mortality did not differ over time for control samples ($p=0.18$). Veliger mortality also did not differ significantly within the barrier at 3-h and 20-h following the start of Zequanox SDP treatment ($p=0.65$; Figure 7).

Percent mortality of adult zebra mussels settled on substrates differed between control locations (outside of the barrier) and locations within the area enclosed by the barrier, and also varied with respect to water depth and distance from Zequanox SDP application points inside the treatment area. At 1-d post-treatment, settled mussel mortality averaged 0.8% in control samples, 82.9% at locations < 5 m from Zequanox SDP application points, 11.1% at locations > 5 m from application points, and 73.6% at locations shallower than application points (Figure 8); mortality was significantly higher at locations < 5 m from application points and in shallow areas within the area enclosed by the barrier compared to control sites ($p<0.05$). The same pattern of highest percent mortality at locations < 5 m from Zequanox SDP application points, lowest percent mortality at control sites and at locations >5 m from Zequanox SDP application points, and intermediate percent mortality for shallow water locations within the area that had been enclosed by the barrier was also observed at two weeks post-treatment ($p<0.05$; Figure 8). Mean mortality for all settled zebra mussels within the treatment area reached 92.7% (±4.1% SE) two weeks after Zequanox SDP treatment, whereas mean mortality for control sites was only 0.23% (± 0.2% SE) at two weeks post-treatment. Similar trends were observed in mussels contained in chambers. Percent mortality of adult zebra mussels contained in the treatment area after 48 days of monitoring were highest in chambers within 5-m of application points (98.8% ± 0.1% SE) and lowest in chambers in waters shallower than application points (55.5%±1.2% SE). Mortality was 0.0% in untreated control chambers.

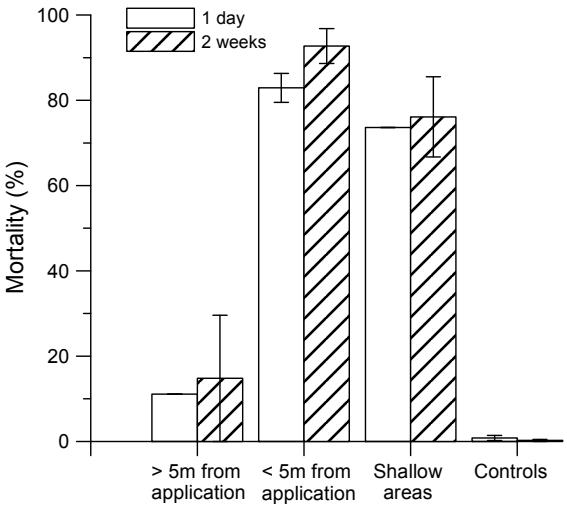

Figure 8. Mean percent mortality (± SE) for zebra mussels in control locations (outside of the barrier) and in relation to distance from treatment areas within the barrier at one day and two weeks post-treatment for the 2013 trial. "Shallow areas" were locations near the lake shoreline within the barrier that were at shallower depths (<0.6 m) than Zequanox® application points.

Discussion

Results indicated that Zequanox SDP was highly effective at killing zebra mussels in application locations in Deep Quarry Lake, with >90% mortality observed within 1–2 weeks of product treatment for mussels attached to substrate and within 3 hours of the start of Zequanox SDP treatment for veligers. Zebra mussel mortality in application sites at Deep Quarry Lake was similar to that observed in field trials for enclosed systems at the Zequanox active ingredient concentrations applied in this study (Rackl et al. 2012) and higher than adult zebra mussel mortality in a contained section of a canal in Ireland that was treated with

Zequanox SDP (Meehan et al. 2014). The slightly higher mortality observed for zebra mussels in containment chambers compared to those of mussels attached to natural substrates was likely due to the longer monitoring periods for contained mussels post-treatment. Mortality of settled mussels was monitored 1 week and 2 weeks while contained mussels were monitored for 2 weeks and 7 weeks in 2012 and 2013, respectively. Zebra mussel mortality typically continues to occur for several weeks after exposure to Zequanox SDP, albeit at a decreasing rate after 1–2 weeks post-treatment in the water temperatures experienced during 2012 and 2013 trials (MBI 2012b). Results of the 2012 trial also indicate that there was no effect of substrate type on the efficacy of Zequanox SDP and that the product was effective for killing all sizes of mussels that were present in treatment areas. Collectively, results of these first open-water trials with Zequanox SDP suggest that application of this product represents a very promising technique for potentially controlling settled zebra mussels in shallow-water habitats in lakes and that Zequanox SDP can effectively kill zebra mussel veligers in treated sections of lakes when product dispersal is somewhat limited as it was in the 2013 trial.

Even with the targeted benthic application method used in the 2013 trial at Deep Quarry Lake, Zequanox SDP dispersed within the deeper areas (>0.6 m depth) inside the barrier, and adult mussel mortality was >90% in locations up to 5 m away from application points. These results indicate that benthic applications from strategically spaced injection points can result in effective treatment of areas up to 5 m from injection points without significant dispersal into the upper layer of water as experienced in 2012. This suggests that whole water column treatments may not be necessary and the overall amount of product applied can be reduced by only applying enough product to treat the bottom layer of water. While the rapid spread of product from injection points indicates that containment of treatment areas is recommended to better maintain product concentrations, applicators do not need to pass application hoses (or other equipment) over the entire targeted treatment area (such as in the 2012 trial) for effective treatment of zebra mussels in lake habitats where control is desired. However, additional study of product dispersal under various environmental conditions (e.g., wind, substrate, water currents) would be useful for development of application protocols tailored to a particular setting. Zequanox SDP did not disperse effectively into locations <0.6 m deep within the containment barrier during the 2013 trial, likely due to lack of an onshore wind during the treatment period and obstructions such as cobble substrate. Limited product dispersal into the shallowest portions of the contained area resulted in significantly lower mortality of both naturally settled and contained zebra mussels in these areas compared to deeper locations within 5 m of Zequanox SDP application points. Thus, our results suggest that Zequanox SDP should be applied to the shallowest areas of the lake littoral zone where treatment of zebra mussels is desired.

While temporary reductions in dissolved oxygen were observed in treatment locations the morning following Zequanox SDP treatment, dissolved oxygen concentrations held at near control levels during the treatment period in both the 2012 and 2013 trials. Dissolved oxygen concentrations quickly rebounded to levels consistent with control sites upon removal of barriers. This suggests that the low dissolved oxygen levels may be avoided through other treatment management practices such as by removing the barriers sooner after treatment, allowing well-oxygenated water from adjacent areas in the lake to circulate into treatment zones. Using a product containment method similar to that used in our study, Meehan et al. (2014) observed higher mortality of adult zebra mussels in aerated enclosures (dissolved oxygen > 7 mg/L) treated with Zequanox SDP compared to enclosures that were not aerated (in which dissolved oxygen declined to 2.38 mg/L 24-h following Zequanox SDP treatment), suggesting that temporarily low dissolved oxygen levels alone were not likely responsible for zebra mussel mortality during the 2012 trial, in which minimum observed dissolved oxygen concentration was 2.5 mg/L. However, we cannot rule out the possibility that temporary low dissolved oxygen concentrations may have contributed to zebra mussel mortality, particularly in the 2013 trial when dissolved oxygen dropped as low as 0.4 mg/L. While low dissolved oxygen concentrations could potentially be harmful to non-target organisms, we observed several juvenile largemouth bass that had been trapped inside treatment plots for 24 h in the 2012 trials that showed no visible ill effects from the Zequanox SDP treatments. We also did not observe any dead macroinvertebrates (crayfish, amphipods, snails, or aquatic insects) in substrate samples collected for estimating zebra mussel mortality, although mortality for non-target taxa was not quantitatively assessed.

Future field applications of Zequanox SDP should evaluate strategies to limit the potentially harmful effects of temporary but substantial reductions in dissolved oxygen on non-target fauna. In addition to earlier removal of barriers following Zequanox SDP treatment as discussed above, potential negative impacts of temporary dissolved oxygen reduction following Zequanox SDP application could also likely be minimized by sequential treatment of relatively small sections of a lake over time rather than treatment of a larger volume of the lake (e.g., up to 50% of lake volume as specified in the EPA label for use of Zequanox in open water systems) in a single application. The former approach is commonly used when applying herbicides to control excessive biomass of aquatic macrophytes.

This study was the first to evaluate Zequanox SDP for as a potential method for controlling zebra mussels in open water systems. Since the commencement of this project, other studies have evaluated other open water uses of Zequanox, for example in an Irish canal (Meehan et al. 2014) and for use in native unionid mussel restoration efforts. There remain several avenues for future research that would be particularly useful to further understanding of the efficacy (including cost-effectiveness), potential ecological impacts of Zequanox SDP application in lakes, and further recommendations for specific invasive mussel management goals. Additional trials could be conducted in lakes with different limnological characteristics than Deep Quarry Lake so that treatment protocols can be tailored to particular types of environments (e.g., water chemistry, lake morphometry and physical habitat). While our results indicated that Zequanox SDP was effective in killing >90% of attached zebra mussels and veligers in treatment areas during mid-summer at Deep Quarry Lake and mortality rates of zebra mussels exposed to Zequanox increase at warmer temperatures due to higher feeding rates (Molloy et al. 2013a), control of zebra mussels may be desired at other times of year, such as during late spring before water temperatures increase to levels that initiate zebra mussel reproduction. Future studies could therefore also investigate the most effective seasonal timing of Zequanox application for rapid and sustained control of zebra and quagga mussels in treatment locations. Determining the frequency of Zequanox applications that will be required for long-term, sustainable control of zebra mussels in locations where treatment is conducted will be particularly useful to lake managers given the high reproductive rates of zebra mussels and potential costs associated with increasing treatment frequency. Complete eradication of well-established zebra mussel populations is probably unlikely in most lakes due to zebra mussel reproductive capacity and potential logistical and fiscal constraints of applying the large quantity of Zequanox SDP that would be required to treat an entire lake volume. However, substantial reductions in zebra mussel densities in targeted, high-value locations such as important habitat for native species or recreational areas within lakes appear to be possible with this product. Additional studies are also recommended to determine effectiveness of Zequanox as a rapid response tool for preventing zebra mussels from establishing a population in a newly infested water body if treatments are conducted during the early stages of an invasion when zebra mussel distribution is limited within a lake and eradication or substantial control of population growth may still be feasible. Finally, future studies should also be conducted to evaluate ecosystem level effects of zebra mussel reduction in lakes in which Zequanox has been applied, such as changes in phytoplankton, zooplankton, benthic invertebrate, and fish biomass and community structures and shifts in food web structure, energy flow, and nutrient cycling, both at a localized level for limited-area treatments and at a larger scale where treatment of large sections of lake are feasible to meet fiscal, recreational, and/or ecological lake management goals. Such larger-scale applications will be possible given recent EPA approval for the Zequanox label to include “recreational and environmental rehabilitation” open water uses that will allow larger treatment areas than were used in this study.

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