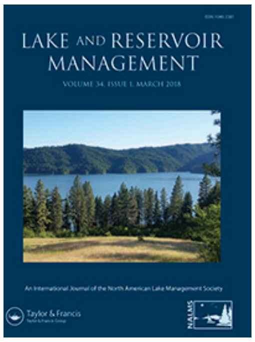

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Zebra mussel (*Dreissena polymorpha*) eradication efforts in Christmas Lake, Minnesota

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ABSTRACT

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In August 2014, an early-detection program discovered a new infestation of zebra mussels (*Dreissena polymorpha*) in Christmas Lake, a small (1.072 km^2) lake near the Twin Cities, Minnesota. Initial surveys suggested a small introduction localized near a public boat access, prompting a rapid response from local and state partners. In 2014–2015, 7 treatments (areas from 243 m^2 to 41,000 m^2) were made with 3 different molluscicides (Zequanox, EarthTec QZ, potash); each used in few prior efforts in open waters. Toxicity bioassays (mussels caged on site and in aquaria) were used to help guide treatments. Intensive SCUBA belt transect and settlement sampler surveys up to one year post-treatment showed that of the ~5500 mussels in the first treatment area (and 10 found just outside it in May 2015), no survivors were recovered. Yet despite rapid coordinated response, in October 2016, 16 mussels were found on structures removed from untreated sites across the lake. The range of shell lengths suggested a remnant population whose larvae had dispersed and settled in scattered locations, and/or dispersal of juveniles from the infestation site. Lessons from this 1 yr eradication attempt highlight the challenges with partial-lake treatments: locating mussels at low densities, containing them within treatment areas large enough given detection uncertainty, and maintaining lethal molluscicide concentrations. Nevertheless, new understanding of these issues, and experience with toxicity and dosing protocols will advise future work. This case study demonstrates the importance of early detection, immediate responses, post-treatment monitoring, and effective cooperation among partners.

KEYWORDS
Dreissenid mussel; early detection/rapid response; EarthTec QZ; potash; Zequanox

Zebra mussels (*Dreissena polymorpha* Pallas 1771) are small freshwater bivalves native to Black, Azov, and Caspian Sea drainages of southern Russia and the Ukraine (Stepien et al. 2014). They were first discovered in the Laurentian Great Lakes in 1988 (Lake St. Clair: Herbert et al. 1989) and have since spread throughout the United States and Canada—reaching >900 water bodies by 2010 (Benson 2014). Most attempts to limit spread have emphasized prevention through watercraft inspection and decontamination of recreational boats and equipment (e.g., trailers, docks, and boat lifts). Management and control of invaded water bodies, in contrast, has been attempted in only a handful of cases, using chemical pesticides (OAFB 2009, DFO 2014, Fernald and Watson 2014) or physical removal (Wimbush et al. 2009, Hargrave et al. 2012).

Zebra mussels were likely first introduced into Minnesota in Duluth/Superior Harbor in 1989. They continued to spread inland via major river systems (Mississippi and St. Croix Rivers and tributaries) over the next 5 yr (Benson 2014) followed somewhat later (starting 2003) by their colonization of inland lakes. In December 2016, the Minnesota Department of Natural Resources (MN DNR) confirmed 132 water bodies (inland lakes, reservoirs, streams, river reaches, and riverine lakes) to be infested with zebra mussels, and listed another 143 as infested due to short waterway connections between them and confirmed infested waters (http://www.dnr.state.mn.us/invasives/ais/infested.html).

In 2010, zebra mussels were first found in Lake Minnetonka, one of the highest recreational-use lakes in Minnesota. Christmas Lake—a small lake with high water clarity, healthy aquatic plant populations, and valuable lakefront property—has its public access just 300 m from the south-central shoreline of

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Minnetonka. High risk of infestation of Christmas Lake prompted inspections of inbound boats and gate-controlled access during off hours at this access, but despite these measures, in August 2015 about 5500 mussels were found confined to the 270 m^2 area surrounding the boat ramp.

In this article, we describe an attempt to eradicate this new, highly localized zebra mussel infestation in Christmas Lake using partial-lake treatments with mollusk pesticides (molluscicides) as part of a rapid response control effort. The project evaluated the efficacy of 3 molluscicides, previously used in few open water treatments, and developed protocols to monitor product concentration and zebra mussel mortality. These outcomes provided a better understanding of toxicity and methods for effective application, and allowed us to evaluate the feasibility of using these molluscicides for control of zebra mussel infestations in lakes. The findings will inform resource managers of the possible outcomes of such projects for future decision-making.

Study site

Christmas Lake is a small (surface area: 1.072 km^2), deep (maximum depth: 26.6 m), spring-fed lake. It is part of the Minnehaha Creek watershed, which drains into the Mississippi River at Minneapolis (~32 km to the east; Fig. 1). Christmas Lake is oligotrophic (chlorophyll *a*: 2 μg/L, total phosphorus: 13 μg/L, Carlson's trophic state index: 37) with high water clarity (Secchi depth >6 m; MCWD 2014). Lake substrate is primarily sand with abundant aquatic plant growth and supports native mussel populations. The public boat access is located on the north side (Fig. 2) in a partially enclosed bay (~48 m^2, maximum depth 6.1 m) that receives less water exchange and wind action compared to the main lake.

Christmas Lake was regarded to be at high infestation risk due to proximity to Lake Minnetonka and a prevention program was implemented including early-detection monitoring. Minnehaha Creek Watershed District (MCWD), a local unit of government tasked with managing the lake, annually conducted baseline assessments of all aquatic invasive species (AIS), using snorkel surveys and plant and invertebrate sampling, including plankton tows for zebra mussel veligers. A zebra mussel settlement plate was installed at the public access and checked twice a month during the open-water season. No zebra mussels were found prior to or during July 2014 surveys, but in August, MCWD staff found 4 attached to the settlement plate. Upon further inspection, numerous zebra mussels were found on rocks near the access. Findings were reported to the

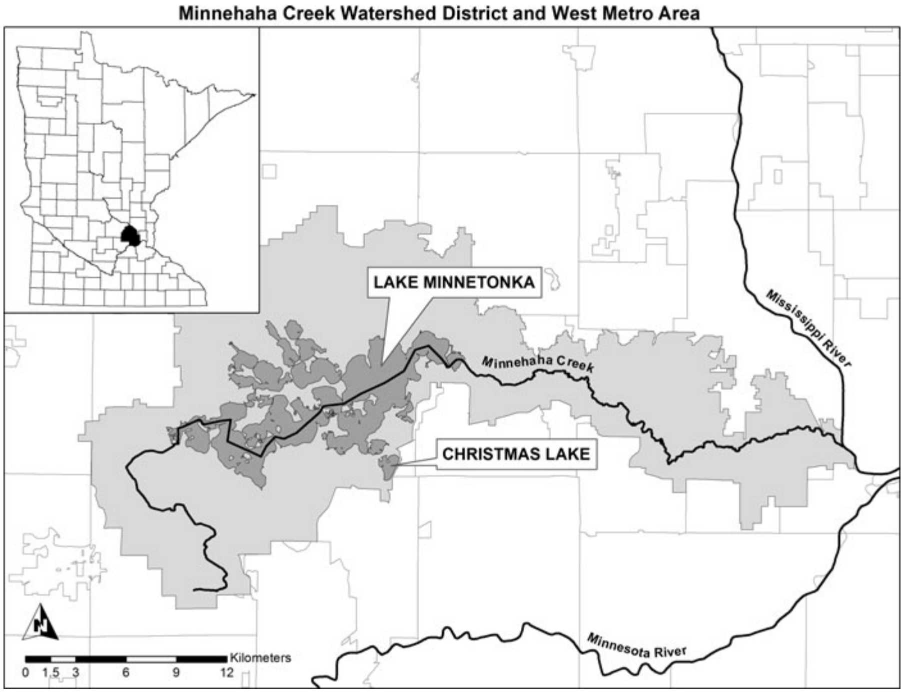

Figure 1. Regional location of Christmas Lake, Hennepin County, MN (inset). Christmas Lake is part of the Minnehaha Creek Watershed, which drains into the Mississippi River at Minneapolis, Hennepin County, MN.

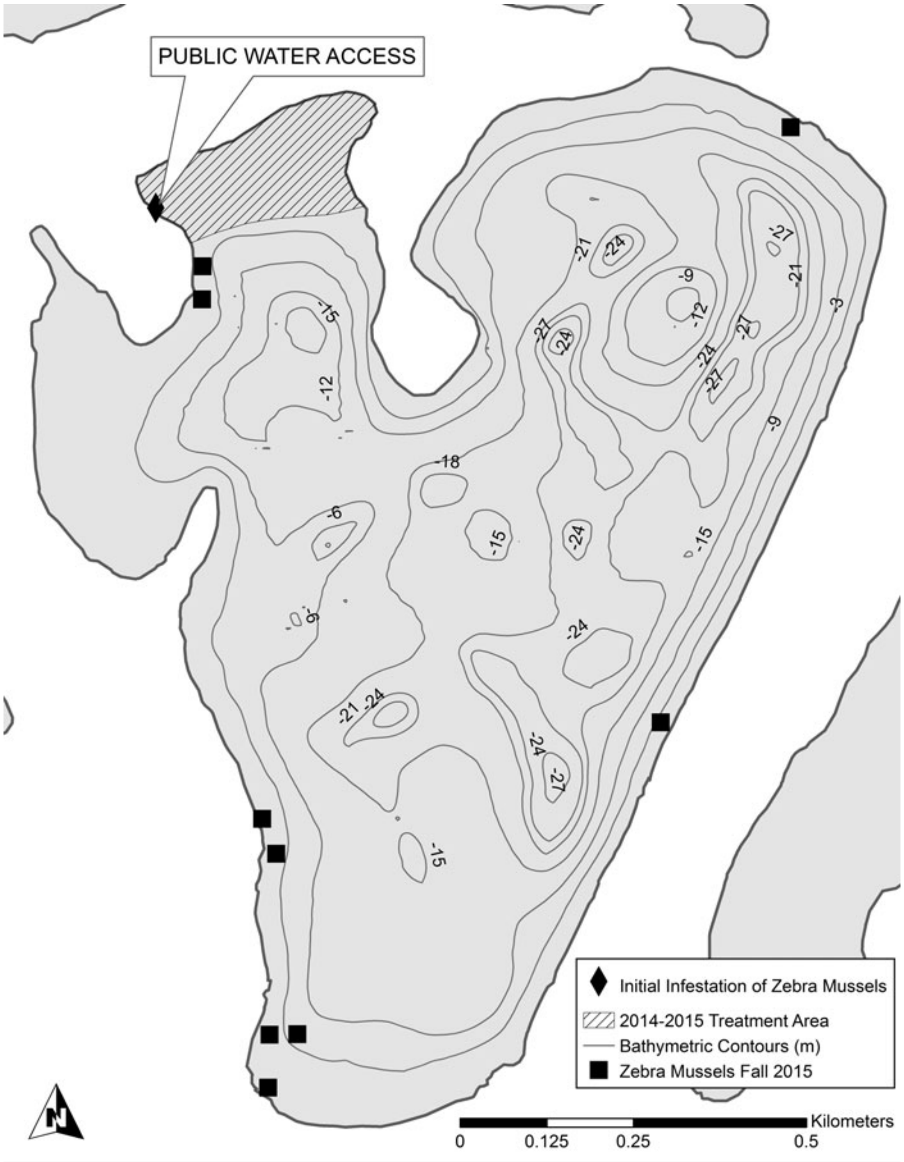

Figure 2. Study site located on Christmas Lake, Hennepin County, MN. Christmas Lake has one public water access located in a northwestern bay. Solid black diamond represents area of initial zebra mussel infestation in Aug 2014. Solid black squares represent the spatial distribution of zebra mussels found during a post-treatment survey in fall 2015. Mussels were discovered on residential docks and lifts during seasonal removal. Depth contours show lake bathymetry depth in meters. Maximum depth of Christmas Lake is 26.5 m.

MN DNR, which organized a lake-wide assessment to determine the extent of the infestation, using snorkel surveys at multiple locations. No additional zebra mussels were found outside the public access area.

Within 4 d of the discovery, a containment barrier (vinyl floating curtain) was placed around the 15 m × 18 m area to which the zebra mussels were confined. Plankton tows were taken at 3 sites across the lake and no veligers were found using cross-polarized light microscopy analysis (Johnson 1995). In the following weeks, a systematic zebra mussel population assessment using SCUBA, snorkel, and wading was conducted within the containment area. Approximately 5500 zebra mussels were found ranging in size from 2 mm to 11 mm (longest shell length). The largest zebra mussel appeared immature with undeveloped gonads and showed no signs of previous spawning upon histological examination (Molloy DP, SUNY University at Albany, Aug 2014, pers. comm.).

A series of stakeholder meetings followed, involving representatives of local and state government, the University of Minnesota, the Christmas Lake Homeowners Association, and lake management consultants. Evidence for a localized population of zebra mussels, its early detection, and lack of evidence for zebra mussel reproduction, together led stakeholders to believe that eradication using molluscicides might be achievable.

Materials and methods

Product selection

Three molluscicide products emerged as viable options for treating zebra mussels in Christmas Lake. Options needed to be selective and capable of achieving 100% mortality of adult mussels. The products selected were (Table 1) Zequanox, EarthTec QZ, and potassium chloride as muriate of potash (hereafter referred to a "potash").

- Zequanox is a biopesticide consisting of the dead bacterial cells of *Pseudomonas fluorescens* strain CL145 A that, when ingested by zebra and quagga mussels, destroy the digestive lining (Marrone Bio Innovations 2015). Only a few in-lake research trials (Marrone Bio Innovations 2012, Luoma et al. 2015a, 2015b) had been conducted using Zequanox prior to this application.
- EarthTec QZ is a copper-based algaecide/bactericide (a formulation of copper sulfate pentahydrate) labeled to control zebra and quagga mussels. The product's active ingredient is delivered in the cupric ion form (Watters et al. 2013). Lethal dose and exposure time of zebra mussels to EarthTec QZ had been identified under laboratory conditions (Watters et al. 2013, Claudi et al. 2014), but had not been explored in the field.
- Potash consists mostly of potassium chloride (KCl). Potassium ions (K^+) interfere with the respiration of zebra and quagga mussels at the gill surface (Fisher et al. 1991, Aquatic Sciences Inc. 1997). Potash is toxic to native mussels but poses a limited threat to aquatic animals other than gill-breathing molluscs (Waller et al. 1993, Aquatic Sciences 1997). Potash is not a registered pesticide in the United States and required a Section 18 Pesticide Emergency Exemption from the US Environmental Protection Agency (USEPA) to allow its use in Minnesota.

EPA permitting

A 24(c) Special Local Needs (SLN) registration was requested for use of EarthTec QZ to control zebra mussels in Christmas Lake. The MN DNR worked with Earth Science Laboratory (product manufacturer of EarthTec QZ) to submit a request to the Minnesota Department of Agriculture (MDA). The SLN registration was requested to allow use of EarthTec QZ at a greater frequency than that listed on the current pesticide label, which requires >14 d pauses between successive treatments. Our initial monitoring showed rapid declines of residual copper within the lake within 24 h. The SLN registration allowed for "bump treatments" to be applied every 2–4 d to maintain toxic doses (see Results). Whether this application frequency will be the norm for future treatments in lakes is a subject for research and in-lake trials (e.g., the EarthTec QZ label provides for lower-dose applications for longer exposure times, which may be easier to maintain without the need for such frequent bump treatments). The SLN registration process took 24 d and was completed by the MDA in early November 2014.

A USEPA Section 18 Emergency Exemption was issued for use of potash to control for zebra mussels in Christmas Lake. The MN DNR worked with MDA to submit a request to the EPA. The permit process took 63 d and was approved for use in December 2014.

Table 1. Product label information on Zequanox, EarthTec QZ, and potash for control of zebra mussels.

Product	Manufacturer	Agent (active ingredient)	Target concentration and exposure time	Concentration and exposure time achieved
Zequanox	Marrone Bio Innovations	*Pseudomonas fluorescens*, strain CL145 A (cell walls of dead bacterial cells)	100 ppm = 98.3 +/− 2.1 NTU[b] (for adults) and 50 ppm (juveniles/veligers) for 8 h	98 NTU (11 d)
EarthTec QZ	Earth Science Laboratories	Copper sulfate pentahydrate(cupric ion [Cu^{++}])	1 ppm (adults) 0.18 ppm (juveniles/veligers) as metallic copper for 96 h	~1 ppm, 14 d (with bump treatments every 2–4 d)
Potash	Mosaic Company	Potassium chloride (K^+)	100–200 ppm of KCl (or 100 ppm K^+) for 48 h[a]	89.3–106.5 ppm, 10 d (41,000 m^2 zone)

[a] Waller et al. (1993) and Fisher et al. (1991).

[b] Target turbidity in NTU (Nephelometric Turbidity Units) = 98.3 ± 2.1 NTU. This was based on a calibration (for 100 ppm active ingredient) in Christmas Lake water, constructed on site on the day of the Zequanox treatment. Mean final turbidity at 11 d post-application was 98 NTU (C. Link and M. Weber, Marrone Bio Innovations).

Product application and monitoring

For partial lake treatments, the use of barriers between the treated and untreated areas is essential. The floating vinyl barriers presented challenges for deployment and maintenance through variable weather conditions. Barriers exposed to wave and wind action were anchored using sandbags and cinder blocks on the windward side. All barriers traversed the entire water column, with the upper edge extending above the water surface. Their deployment allowed for greatly diminished mixing between treated and untreated waters and helped maintain target concentrations over longer intervals between bump treatments. Target concentrations could not have been achieved and maintained as stable as they were over time without barriers.

Monitoring protocols were developed to assess in-lake molluscicide concentrations. During application, concentrations were measured to ensure that target concentrations were achieved to kill zebra mussels and these concentrations were maintained via bump treatments. All products were applied in Christmas Lake by a state-certified pesticide applicator. For larger treatments (20–40 m^2), morphometric analysis of water volume was calculated using sonar data and analysis from BioBase (Navico Inc.) in order to calculate product dosing prior to application.

Products were mixed in tanks and injected at the water surface. Following treatment, monitoring occurred every 1–2 d for 14 d post-treatment. Monitoring consisted of collecting surface water samples at various locations inside the treatment area. Samples were submitted to Minnesota Department of Agriculture laboratory for analysis by mass spectroscopy, with results reported within 1–2 d. Portable meters (e.g., LaMotte 1200 Colorimeter for Cu^{2+} concentrations and YSI 9500 Photometer for K^+ concentrations) were used to inform bump applications in the field.

During the Zequanox application, concentrations were estimated, using turbidity measurement, on the first and last day of treatment application. Monitoring of concentrations more often is of limited utility, since evidence indicates that the active agent in Zequanox is degraded within 24 h after it is added to water (Molloy et al. 2013). Zebra mussel mortality was assessed via in-lake cage bioassays. Four cages of ~50–100 mussels per cage were placed within the treatment area. Cages were constructed of plastic canvas mesh sheets (1–2 mm openings), anchored to the lake bottom. Live, gaping, and dead zebra mussels were recorded daily until all mussels were dead or until no additional mussels died over 3 consecutive days.

Laboratory product efficacy testing was conducted in tandem with in-lake applications for Zequanox, EarthTec QZ, and potash. Zebra mussel mortality was assessed via aquarium bioassays. Bioassays conducted in the laboratory provided an independent evaluation of toxicity of the treated lake water under conditions allowing greater control and simplifying the scoring of mortality events. Water was collected from the treatment area prior to (control aquaria) and immediately following (treatment aquaria) molluscicide applications. Each sample of water was divided among three 76 L aquarium tanks, totaling 6 tanks (3 treatment, 3 control). Zebra mussels in the bioassays were collected from Christmas Lake (Zequanoxtrial) and Lake Minnetonka (EarthTec QZ and potash trials; due to scarcity of zebra mussels in Christmas Lake at the time of these treatments). Fifty zebra mussels were placed in each tank and enclosed in acrylic tubes with 2 mm nylon mesh covering the open ends. Zebra mussels were allowed to acclimate in tanks for 1–2 d prior to exposure. Temperature and dissolved oxygen (DO) concentrations were monitored during each trial. Live and dead zebra mussels were scored daily and dead mussels were removed. Trials ended when all mussels were presumed dead or until no mussels died after 3 consecutive days. For EarthTec QZ and potash, aquarium trials were also conducted on untreated lake water dosed with varying concentrations of the agent in the lab.

Post-treatment monitoring

Search efforts were conducted after each treatment in an attempt to find any live or dead zebra mussels in the treated area and beyond. Areas were searched inside the treatment area, within the immediate proximity of the treatment area (15–30 m buffer), and at a lake-wide scale targeting ideal settlement areas (i.e., areas with hard substrates, suitable depths [0.5–4 m], and often occupied by native freshwater mussels). Belt transect surveys (30 m transect line) parallel to shore were conducted regularly using SCUBA, snorkel, and wading. Lastly, a comprehensive search of the entire shoreline was also conducted by 18 surveyors using both SCUBA and snorkel gear. In addition to active searches, settlement samplers (4 stacked grey

PVC plates, 15 cm × 15 cm) were suspended from docks and buoys at several locations around the lake perimeter. Samplers were checked for juvenile zebra mussels periodically throughout the 2015 summer and removed in the fall. Samplers were monitored by MCWD and lake homeowners.

Results

Treatment summary narrative and molluscicide efficacy

First we provide the chronology of treatment efforts to control zebra mussels in Christmas Lake (summarized also in Fig. 3). In September 2014, Christmas Lake was treated with Zequanox 23 d after the initial infestation was detected. This was the first time Zequanox had been applied in open water for zebra mussel management efforts (i.e., in a non-research application). The area treated was 15 m × 18 m (243 m^2) at the public boat access. Marrone Bio Innovations personnel monitored product concentration the day of treatment until the target concentration of 100 ppm active ingredient was achieved, estimated using turbidity measures that on Day 1 were 98.3 ± 2.1 Nephelometric Turbidity Units (NTU). Turbidity by Day 11 was still 98 NTU. Dissolved oxygen and zebra mussel mortality was monitored by the MCWD following treatment. Average water temperature in the treatment area was 17.4 C. Dissolved oxygen dropped from 7.81 mg/L to 0.1 mg/L within the first 24 h. Based on estimates from cage and aquarium bioassays, we achieved 100% mortality by Day 11 (dissolved oxygen measurements were 4.35 mg/L in cages and 0.5 mg/L in aquaria; Fig. 4). No mortality was observed in the control tanks (dissolved oxygen range: 7.36–8.91 mg/L). Additional searching following treatment found 25 additional zebra mussels 9–18 m outside the treatment area.

From October to November 2014, a series of treatments using EarthTec QZ targeted the initial infested area and the area surrounding the location of the 25 newly discovered zebra mussels. The maximum area treated with EarthTec was 3035 m^2. In-lake copper concentrations were measured and aquarium bioassays on treated lake water were conducted (because mussels were too scarce to allow mortality bioassays to be conducted in the lake). During the initial EarthTec QZ treatment, copper concentrations decreased from 1 ppm to 0.5 ppm within 8 h. No barrier was used during this treatment and only one application was allowed every 14 d as per the existing product label. An expanded barrier was installed during the second

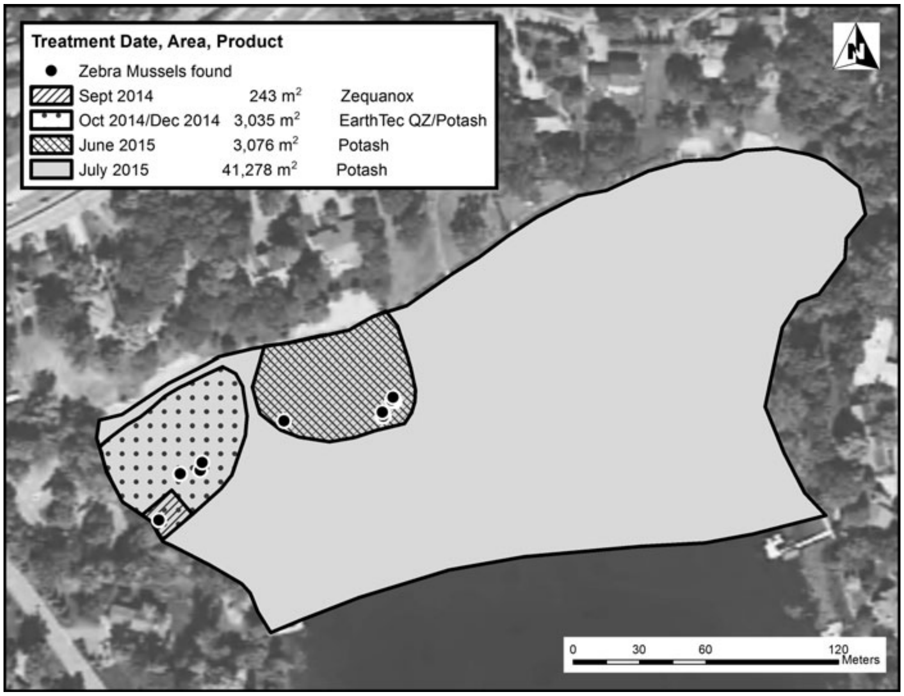

Figure 3. Treatment history of zebra mussel control efforts in Christmas Lake, Hennepin County, MN. Date of treatment (month and year), dimensions of area treated, and products used during treatment are provided (upper left table). Black outlines enclose areas treated. Solid black dots show where zebra mussels were found prior to treatment.

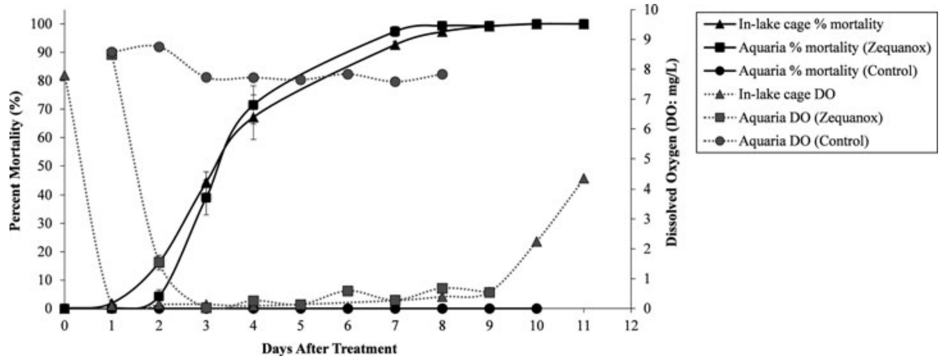

Figure 4. Percent mortality of zebra mussels (solid black symbols) exposed to Zequanox during aquarium trials and in-lake cage trials. In-lake trials were conducted in Christmas Lake, Hennepin County, MN. Mean water temperature was 18 C. Values plotted are means and error bars represent one standard error. DO = dissolved oxygen concentrations (mg/L).

EarthTec QZ treatment in an attempt to maintain longer exposure durations at lethal concentrations. Following treatment, copper concentrations dropped below 0.5 ppm within 96 h, still insufficient to allow for 100% zebra mussel mortality. The 24(c) Special Local Needs registration allowed for more frequent treatments to maintain desired concentrations. EarthTec QZ was used in Christmas Lake for the third time, this time applied every 2–4 d in order to maintain necessary lethal concentrations for a period of 14 d. Unexpected ice formed within the treatment area by Day 10 and as result terminated scheduled applications for the remainder of the treatment. Still, we found that copper concentrations remained between 0.6–1 ppm for 10 d, and were still at 1 ppm for the additional 4 d after ice formation, extending into Day 14. Copper concentrations slowly decreased under the ice, but were still at 0.2 ppm 30 d post-treatment. Water temperatures ranged from 1 to 2 C during the treatments, prior to ice formation. Copper concentrations in aquarium trials of lake water taken from the treatment area (at the time of the 3035 m^2 treatment) ranged from 0.6 to 0.9 ppm. Mortality of 100% was observed in these trials in 6–8 d. No mortality was observed in the control trials. A subset of tanks was also dosed in the lab with 0.5 ppm and 1 ppm of EarthTec QZ to reevaluate toxicity over a range bracketing concentrations in the lake. Zebra mussel mortality of 100% was observed by Day 7 at both 0.5 ppm and 1.0 ppm (Fig. 5).

In December 2014 and June/July 2015, Christmas Lake was treated with potash after receiving USEPA approval for a Section 18 Emergency Exemption. The area treated in December was 3035 m^2; using the existing barriers (which were frozen in place), the potash was injected under the ice (100 ppm K^+). This was the first time potash has been used for zebra mussel control in Minnesota and the first time potash had been applied underneath ice. Potassium (K^+), chloride (Cl^-), and conductivity were monitored during and after treatment for 14 d to ensure target concentrations were being met. Concentrations of potassium were variable within the treatment area, 3.36–490 ppm, indicating an uneven horizontal and vertical mixing of product. This was likely due to colder water temperatures (4 C) and the resulting higher product solution density, and perhaps to the absence of wind-driven

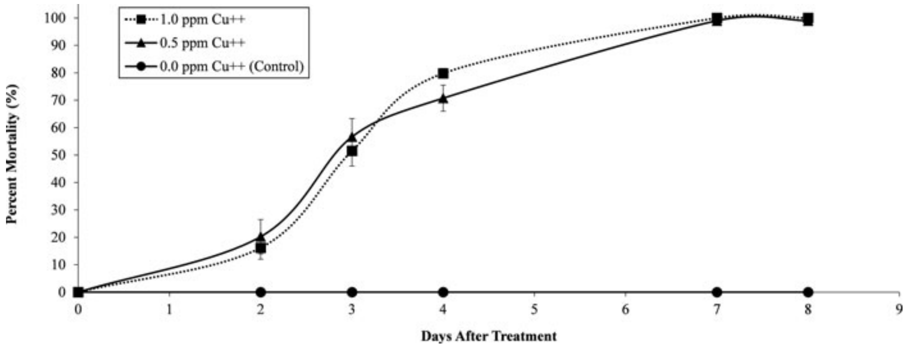

Figure 5. Percent mortality of zebra mussels in aquarium trials when exposed to EarthTec QZ at the doses shown. Mean water temperature was 16.5 C. Values plotted are means; error bars represent one standard error.

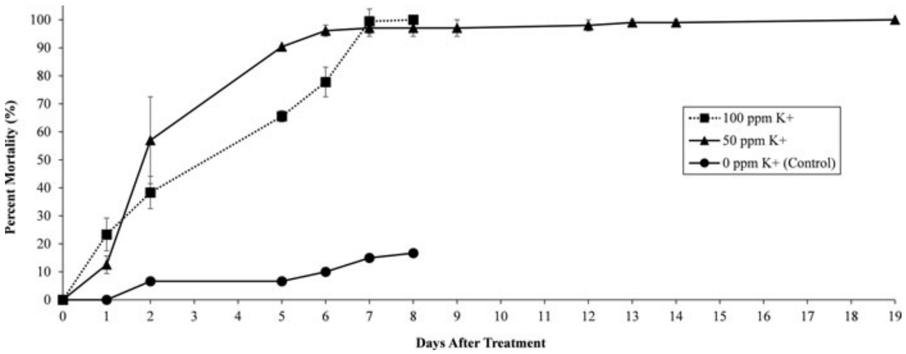

Figure 6. Percent mortality of zebra mussels when exposed to potash in aquarium trials. Mean water temperature was 1 C. Values plotted are means; error bars represent one standard error.

circulation of lake waters as well. Chloride and conductivity showed similar patterns (Cl^- concentrations ranged from 51 to 424 ppm and conductivity ranged from 471 to 1572 µS/cm). For aquarium trials, 100% mortality was observed by Day 7–19 in these initial trials (data not shown), in which water temperatures ranged from 16 to 17 C in the tanks, considerably warmer than in-lake temperatures. Aquarium trials were then repeated with cold water (maintained at 1 C average across tanks). These cold-water tanks were treated at the same concentrations (50 ppm and 100 ppm K^+) as in the 16–17 C trials. In these colder 1 C trials, 100% mortality was achieved by Day 9 in the treated tanks, and 17% (mean) mortality was observed across the control tanks (Fig. 6).

Following ice-off on Christmas Lake, post-treatment assessments occurred in April and May of 2015. In May 2015, an extensive lake-wide search found 10 zebra mussels attached to native freshwater mussels outside of previously treated areas at distances ranging from about 10 to 50 m from the previous containment barrier's edge. A 3076 m^2 area was contained and treated with potash in June. Concentrations of potassium ranged between 73.5 and 110 ppm K^+ over 10 d. Stakeholder concerns remained regarding effective treatment size and a meeting was convened where an expansion of past treatment areas was considered. A proposal was made to enclose all previously treated areas in a zone that spanned much of the adjoining bay. Rationale for this proposal included the inadequacy of past efforts to sufficiently locate and enclose all zebra mussels within a treatment area, as well as risk associated with warming water temperatures that could cue spawning events of any undiscovered adult mussels. After discussion and debate, the MN DNR approved the proposal; a 41,000 m^2 zone was delineated using containment barrier and treated with potash on 27 June 2016. Bump treatments occurred on Day 7 to maintain concentrations to within lethal-range levels, and the barriers were left in place for 12 d. Across the entire treatment duration, potassium concentrations ranging from 89.3 ppm to 106.5 ppm were recorded in surface waters.

Summary of total zebra mussel monitoring and survey efforts

Time and expense for such rapid response actions were considerable but not excessive, in the light of other past AIS eradication efforts (Discussion). Roughly $64,000 in direct cost (excluding salaries and fringe for personnel) was expended between the MCWD and MN DNR throughout this project with one-third of the cost coming from the final 41,000 m^2 potash treatment. The Christmas Lake Homeowners Association expended additional resources in the form of technical consultation and by providing temporary access for homeowners during treatments. Staff from MCWD and MN DNR dedicated over 520 h in the field and laboratory to this project and an additional 88 h were contracted with consultants.

Final zebra mussel searches

After the final 27 June potash treatment, monthly zebra mussel searches occurred in July, August, and September of 2015. Searches consisted of 2–5 divers examining multiple areas around the lake either using snorkeling or SCUBA gear. Zebra mussel sampler plates were checked weekly at the public access dock, and 13 volunteer homeowners had zebra mussel sampling plates attached to their docks in various locations on the lake.

After over a year of extensive efforts to eradicate zebra mussels in Christmas Lake, 16 zebra mussels were found attached to docks, boat lifts, and sampler plates in untreated areas in October 2015 (Fig. 2). In 2016, successful reproduction and settlement was confirmed to have occurred, with juvenile and adult zebra mussels being found at multiple locations outside the treatment areas. The MN DNR and MCWD agreed that no further treatment efforts would occur on Christmas Lake.

Discussion

The 2014–2015 molluscicide treatment efforts on Christmas Lake had the following management objectives: (1) to evaluate the efficacy of candidate molluscicides for treating zebra mussels in open waters, (2) to create a set of working protocols for monitoring molluscicide concentrations and zebra mussel mortality, and (3) to eradicate zebra mussels from Christmas Lake. Which of these objectives were met and what was learned from these efforts?

Evaluation of molluscicide treatments in open water

Using molluscicides with so few prior attempts in open-water treatments presented challenges in terms of both product selection and application protocols. The use of both Zequanox and EarthTec QZ represented, to our knowledge, the first in-lake (non-research) applications for both molluscicides. Observations from laboratory experiments provided the needed target concentrations and exposures, and all 3 molluscicides utilized in our efforts were shown to be lethal either from in-lake bioassays or laboratory exposures. Yet achieving target concentrations and maintaining lethal exposures over time was a lesson in adaptive management. For these partial-lake treatments, the installation of curtain barriers was absolutely required to maintain lethal concentrations over the necessary exposure durations. In addition to this, a number of factors such as declines in dissolved oxygen, variable water temperatures, and size of treatment areas influenced the success of our efforts.

Maintaining curtain barriers in place during the Zequanox treatment resulted in declines in dissolved oxygen. This was clearly a factor that contributed to zebra mussel mortality, and future work should monitor dissolved oxygen declines and attempt to quantify their contributions to mortality, relative to toxicity of the treatment agent. The treatment area on Christmas Lake reached dissolved oxygen values of 0.1 ppm within 24 h, and these hypoxic conditions continued through Day 7, when mussel mortality had reached 90%. Laboratory studies have revealed that zebra mussels are relatively sensitive to hypoxia, but time to mortality results have varied substantially between studies. European zebra mussels reached 100% mortality within 144 h (6 d) at the same temperatures (17–18 C) present within Christmas Lake during the Zequanox treatment, when the sealed vessels housing these mussels were completely depleted of O_2 by mussel respiration (Mikheev 1964). However, survival times of North American mussels (from the Niagara River) were somewhat longer (228–428 h [9.5–17.8 d]) when anoxia was achieved by bubbling their holding chambers with N_2 gas (McMahon 1996). Much shorter survival times in Mikheev (1964) may have been the result of build-up of toxic anaerobic metabolic products, and therefore not as relevant to the outcome we obtained inside the lake enclosure. Accounting for the effects of hypoxia on zebra mussels and potential effects of hypoxia to nontarget organisms are important considerations for evaluating molluscicide agents for future dreissenid response efforts. Dropping curtain barriers 24 h after treatment could be one solution in the case of Zequanox that could reduce nontarget effects and allow for clearer interpretation of Zequanox toxicity, but the risk will be the loss of containment of any enclosed mussels that were not killed by the treatment.

Reduced water temperatures also had a sizable impact on methods used and outcomes from our molluscicide applications. Zequanox was not recommended by the manufacturer for late fall 2014 treatments due to reduced zebra mussel feeding and metabolic rates in colder waters (because the agent has to be ingested to be active). Copper concentrations in the EarthTec QZ treatment (fall 2014) may have remained higher for a longer duration, due to reduced water temperatures (ranging from 1 to 2 C) and to slower uptake by biota and/or degradation of the agent in the water column at colder temperatures. The initial potash application (winter 2014/2015) of course faced new logistical challenges of treating zebra mussels under the ice. A related and unanticipated problem came from the fact that the applied potash solution is denser than lake water, which resulted in K^+ concentrations that varied throughout the water column (from 3.36 to 490 ppm) with “hotspots” accumulating at the deepest depths. Attempts to mix treated water under the ice proved largely unsuccessful. Further refinement of application methods in cold-water conditions would benefit from additional field evaluations, and laboratory study of toxicity of molluscicides at these temperatures.

Lastly, lessons were learned about effective treatment area size (i.e., the spatial extent and necessary buffering of presumed isolated zebra mussel populations). Following the initial find of mussels near the public boat access, a very small area (243 m^2) was cordoned off and treated with Zequanox. The area was later expanded upon the discovery of additional mussels just beyond the enclosure. The expanded area (3035 m^2) again was too small when, the following spring, adult zebra mussels were discovered attached to native freshwater mussels along the shoreline immediately adjacent to the 2014 treatment areas. One likely scenario is that these zebra mussels originated from the first infestation site and moved along shore by hitchhiking on native mussels (*Pyganodon grandis* and *Lampsilis siliquoidea*; DNR surveys have also found *Utterbackia inbicilis* in Christmas Lake in smaller numbers: B. Sietman, pers. comm.). Some species of unionids such as *Elliptio complanata*, although not present in this lake, can move considerable distances horizontally as well as vertically in response to various stimuli (Balfour and Smock 1995, Amyot and Downing 1997). To enclose the zebra mussels attached to native mussels the treatment area was expanded, eventually to 41,000 m^2 for the final 27 June treatment. If the "early response, partial-lake treatment" management option is to succeed, we strongly suspect that treatment areas will need to be expanded to account for density and distribution patterns within a lake and for detection uncertainty of surveys (particularly at low zebra mussel population density). Statistical analyses that incorporate uncertainty in spatial distribution of freshwater mussels (e.g., Pooler and Smith 2005) may help establish treatment area dimensions in future attempts.

Creation of monitoring protocols

The development of a set of monitoring protocols to be used for molluscicide treatment projects was a major contribution realized from these eradication efforts. The partners in the Christmas Lake treatment effort recognized, early on, that the project offered opportunities for development and field-testing of procedures for studying the success of zebra mussel molluscicide treatments. Of particular interest was how to design a post-treatment monitoring program to study the response of the lake's zebra mussel population in the years following chemical treatment. Implementing long-term monitoring following zebra mussel molluscicide treatment faces some considerable design challenges and is expensive, and so very little data exists on longer-term effects of zebra mussel molluscicide treatment. Included in the challenges is how to most efficiently survey lakes with very low densities of adult mussels (as is the case for lakes that are candidates for molluscicide treatment), how to use survey results to size treatment areas, and how to design studies to determine if molluscicide treatment causes population size reductions. Our working protocols that came out of the Christmas Lake project were intended to address these issues. Included activities and data to be collected are summarized below.

1. *Estimates of mortality from molluscicide treatment.* Tank bioassays were conducted to evaluate the toxicity of the lots of molluscicides supplied by manufacturers, and for Zequanox, the mixture applied to the lake. These assays were conducted under laboratory conditions so that exposure concentrations and durations could be carefully controlled. In ideal circumstances, tank bioassay is a valuable component of a treatment project with any zebra mussel molluscicide, given the relative scarcity of toxicity data for these agents. An activity of even greater importance is bioassay for toxicity in situ. We found that caged animals placed within the treatment area was a preferred approach, because animals left on the lake bottom that die often detach, and are more difficult to recover and count if not caged.
2. *Adult mussel surveys for abundance and spatial distribution inside the treatment area.* In the treatment area, methods for survey of adult mussels are required to estimate abundance and patterns of spatial distribution. Reliable abundance estimates are necessary to allow assessment of population trends post-treatment, and reliable maps of spatial distribution are required for several reasons—for one, they provide the basis for estimating the dimensions of the treatment area and for placement of the curtain barriers.

3. *Surveys for abundance outside the treatment area.* Outside the treatment area, methods are needed to allow estimates of adult mussel abundance, such that trends in population size, lake-wide, can be tracked after treatment. In Christmas Lake, mussels were located in October 2015 at sites various distances from the initial site of infestation, some far removed. In lakes with mussels remaining post-treatment, reproductive activity in the following season may result in veliger larvae dispersing to distant sites, leading to a more scattered population that may or may not grow in the seasons after treatment is discontinued. Permanent transects placed outside the treatment area are where monitoring for this sort of population response should be focused. This should be supplemented with regular survey of docks and other structures as they are removed from the lake in autumn, as this is an effective way to cover large lake areas far from treatment sites—as demonstrated in Christmas Lake.
4. *Molluscicide residue monitoring and miscellaneous activities.* The treatment protocols contain recommendations for monitoring of molluscicide residues, including recommended residue testing technology. Among the miscellaneous activities that can provide information on lake responses are procedures for monitoring settlement of juvenile mussels and procedures for monitoring of veliger larva concentrations using plankton tows.

Eradication of zebra mussels

The discovery of a few zebra mussels (including juveniles) in the fall of 2015 and numerous juvenile and adult zebra mussels in the fall of 2016 confirmed that eradication had not been achieved. One understandable reaction to this outcome is that eradication was an unrealistic expectation in the first place. This opinion is common, as eradication of invasive species is often claimed to be an implausible goal. However, this suspicion has been challenged (Myers et al. 2000, Simberloff 2001), and in fact numerous cases exist where attempts to eradicate insects, invasive mammals, and marine invasive species (Kuris and Culver 1999, Williams and Schroeder 2004, Anderson 2005) have led to extirpation of the pest (Simberloff 2001, Simberloff 2003). Relevant to the present discussion is the case of the invasive brackish water mussel *Mytilopsis sallei* (a relative of zebra mussels) that was successfully extirpated from a harbor in Darwin, Australia, using aggressive chemical treatments (Bax et al. 2002).

Several small infestations of zebra mussels have been eradicated (or their populations greatly suppressed) in US waters using both chemical and mechanical removal methods. Control has been achieved using molluscicides such as potassium chloride (DFO 2014, Fernald and Watson 2014) and copper sulfate (OAFB 2009) or physical measures such as hand removal via SCUBA (Wimbush et al. 2009) and winter drawdowns (Hargrave et al. 2012). Success stories include a case of eradication by potash treatment in Millbrook Quarry, Virginia. This was a whole–water body treatment—the entire 46 m^2 quarry was dosed with 131,000 kg potash in February 2006 and not a single live mussel has been since discovered (Fernald and Watson 2014). Hand removal of zebra mussels by SCUBA was utilized in Lake George, New York, over an 8 yr period, during which divers harvested approximately 21,000 zebra mussels and little or no recruitment has since occurred (Wimbush et al. 2009). Small isolated outbreaks in bays on Lake George—most likely chronic, localized reintroductions—have been controlled by hand removal and no recruitment has since been recorded (Wimbush et al. 2009).

It may be fruitful at this point to examine the status of the development of zebra mussel management in the light of the history of research and development of aquatic plant management (APM) science. APM has experienced an evolution in methodologies, and likewise, there will need to be an evolution in the methodologies that are developed for the management of aquatic invasive animal species, including dreissenid mussels. This study endeavored to advance this process by using in–treatment zone cages, laboratory tanks, and a variety of developing approaches to applying chemicals in open waters and monitoring their effects. In aquatic plant management attempts, eradication is rarely achieved, though low plant densities can be; population suppression is a realistic goal that is expensive, but it can have tangible benefits. The questions that must be addressed for zebra mussels are similar: Can we suppress populations? How can we best do it? In which cases will the ecologic and economic benefits of suppression outweigh the costs?

Other management implications

Partial lake management efforts like the Christmas Lake project are challenged by dispersal powers of zebra mussels and difficulties with preventing their escape to lake areas outside containment barriers. We observed apparent escape from the treatment site (and probable site of initial colonization) by zebra mussels attached to crawling native mussels. Another possible mechanism for escape is dispersal of veliger larvae from spawning of mussels that were not detected during post-treatment dive and snorkel surveys conducted in 2015, prior to the reproductive season. Research shows that in dreissenid mussels, fertilization success depends on density and proximity between spawning males and females (Quinn and Ackerman 2011, 2012), just like it does in free-spawing marine animals (Pennington 1985, Levitan 1992). This means that isolated mussels missed during surveys would not have been a source for larval dispersal (and there were no clusters remaining in the treatment area), so for this mechanism to be responsible, there must have been reproductive groups of mussels in undiscovered locations. Even in small lakes this is not unexpected.

Other conceivable mechanisms for escape include drifting of "postmetamorphic" (settled) juvenile mussels that detach their byssal threads and are carried by water currents to settle in new locations. Postmetamorphic drifters were abundant on settlement collectors, even in mid-water plankton samples during August sampling in Lake Erie (Martel 1993). Similarly, juvenile and adult mussels can raft on drifting vegetation (Horvath and Lamberti 1997). Either of these mechanisms could have been the source of the larger animals (one measured 18 mm shell length) found across the lake and far from the public access in October 2015. Other mechanisms include crawling of adults (Toomey et al. 2002)—this allows mobility over short distances—and independent introductions, like those founding infestations in small bays in Lake George (Wimbush et al. 2009). Since mussels discovered in October 2015 were on structures not close to public accesses, these latter 2 scenarios seem less likely. Whatever the case, mobility and dispersal powers of zebra mussels in lakes further challenges the use of partial-lake treatments, given <100% detection efficiency and the near impossibility of containing all mussels in a lake inside treatment barriers.

Decision makers in the case of Christmas Lake were stretched to utilize a variety of treatment agents versus one choice agent due to limited options in terms of EPA registered molluscicides, product labeling restrictions, and few data from past in-lake trials to influence eradication prescriptions. This application of different classes of molluscicide in succession did compromise our ability to assign mortality outcomes to any one of the individual agents. Does this make the project useless for research needs? No, because as stated above, we were still able to make progress on open-water toxicity of single agents with bioassays. The Christmas Lake project also points out prospects for a completely distinct research objective. Zebra mussel population response to molluscicide treatment has yet to be assessed in open-water applications. For studying population response, the agent causing a given level of mortality is not the focus—instead the focus is on documenting mortality, and on documenting population trends along with pertinent water quality monitoring post-treatment. This outcome could still be assessed in the case of Christmas Lake and future cases, given sufficient monitoring effort over the coming years. There is a list of questions to be answered: Can population growth be suppressed by pesticide treatment, and how long can suppression be maintained? Is follow-up treatment necessary and at what frequency? Is control of population growth, without complete elimination of mussels from a lake, economically feasible? The opportunity to begin to address these questions exists because extensive surveys were conducted before and immediately following the treatment attempt and provide a baseline.

The Christmas Lake project demonstrated the potential for conflicts arising between demands for progress on management and on research, and points to directions for managing these. Research attempts to examine efficacy of zebra mussel molluscicides would ideally test single agents in controlled assays. So far, for zebra mussel molluscicides, these have been limited to trials in the laboratory and in field enclosures (Costa et al. 2011, Luoma et al. 2015a, 2015b). And so, while open water research on applications remain in great need, this was not the intent of the Christmas Lake project; rather this was a real-time attempt to extirpate an isolated introduction of zebra mussels, from which we learned valuable lessons to guide future dreissenid pesticide treatment efforts.

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