Effects of Spray-Dried *Pseudomonas fluorescens*, Strain CL145A (Zequanox®) on Reproduction and Early Development of the Fathead Minnow (*Pimephales promelas)*

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Abstract

The biopesticide, Zequanox®, is registered for dreissenid mussel control in open water systems. Previous toxicity trials with nontarget organisms, including young-of-the year of several fish species and invertebrates, demonstrated selectivity of Zequanox for dreissenids. However, data are lacking on its safety to reproductive and early life stages of fish. The present study evaluated the effects of Zequanox on spawning and early life stages of the fathead minnow, *Pimephales promelas,* at the maximum approved concentration (100 mg Zequanox active ingredient /L) and exposure duration (8 h) for open water application. The results showed no significant effect of Zequanox on survival, condition, or cumulative egg deposition (21 d) in adult fathead minnow. Eggs (<24-h old) exposed to Zequanox developed to the eyed-stage at a similar rate to that of unexposed eggs. Additionally, Zequanox did not have a significant effect on survival and growth (90 d) of newly hatched fry (<24-h old). The results indicate that Zequanox treatment will not affect survival, spawning, and early life development of fathead minnows when applied at the recommended treatment regime.

Introduction

Zebra mussels *(Dreissena polymorpha* Pallas, 1771) and Quagga mussels, (*D.rostriformis bugensis* Andrusov, 1898) are nuisance invasive species that have expanded their range throughout the United States and into Canada since their arrival in 1985 (Mackie and Claudi 2009). These mussels have adversely affected aquatic communities on a number of levels. The high filtration capacity of dreissenids has caused a shift from a pelagic to a benthic food web, resulting in alterations in diet and concomitant reduction in condition of some fish species (Vanderploeg et al. 2002, Pothoven and Mathajian 2008, Nalepa et al. 2009). Settlement of dreissenids in high densities has caused degradation of fish spawning shoals (Marsden and Chotkowski 2001). Native mussels have declined and disappeared from habitats throughout the Great Lakes' region due to competition for food and colonization by dreissenids (e.g., Nalepa et al. 1996, Ricciardi et al. 1996, Schloesser et al. 1998, Strayer and Malcom 2007). Dreissenid infestations have altered fish management activities by compromising or eliminating sources of brood stock and eggs for aquaculture (OMNR 2005, Sykes 2009).

One potential molluscicide to control dreissenids in open water is the biopesticide,

[1] U.S. Geological Survey.

Zequanox®, a commercial formulation of a ubiquitous soil bacterium, *Pseudomonas fluorescens* strain CL145A. The product has shown specific toxicity to dreissenid mussels and low toxicity to a range of nontarget species (Marrone Bio Innovations [MBI] 2012a, Molloy et al. 2013a). Zequanox is registered by the US Environmental Protection Agency (USEPA) for treatment of dreissenids in closed and open water applications (MBI 2012b; registration number 84059-15). Toxicity tests with bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and brown trout (*Salmo trutta*) showed no evidence of mortality from exposure to 100 mg Zequanox active ingredient (A.I.)/L for 72h (Molloy et al. 2013b). Luoma et al. (2015) tested fingerlings of a variety of warm, cool- and cold-water fish species in a continuous 24-h exposure to concentrations ranging from 50 to 300 mg Zequanox A.I./L. Significant differences in toxicity occurred among species; for example, rainbow trout (*S. gairdneri*) and lake sturgeon (*Acipenser fluvescens*) were highly sensitive (24-h LC50 = 19.2 mg A.I./L and 8.9 mg A.I./L, respectively), while the LC50 value exceeded 100 mg Zequanox A.I. /L for six other species. However, the exposure duration in their trials was three times longer than the expected exposure duration (i.e., 24-h continuous dosing). There are no published studies on the toxicity of Zequanox to fish in static exposures at the maximum approved open-water label concentration (100 mg Zequanox A.I./L) and exposure duration (8h). Additionally, previous trials with fish tested juvenile/fingerling life stages. Data are lacking on the safety of Zequanox to spawning adults, eggs, and early life stages of fish.

Fathead minnows are well-established test organisms for measuring toxicant effects on survival and reproductive fitness of a cyprinids and related species (Ankley et al. 2001; Jensen et al. 2001; Kahl et al. 2001). Reproductive maturity of males and females can be established by the appearance of secondary sex characteristics; males develop a dorsal pad and various sizes and numbers of nuptial tubercles. Fathead minnows are fractional spawners and females may produce clutches of 50-100 eggs every 3-5 days at 25°C (Gale and Buynak 1982; Jensen et al. 2001; Thorp et al. 2007). Fertilized eggs undergo cleavage to the blastula stage within 3-4 h of spawning and can readily be distinguished from unfertilized eggs by an opaque or clear appearance and a white spot where the yolk has precipitated (USEPA 2002). The eyed-stage develops within 48 to 72 h (USEPA 1996) and can be used to distinguish unfertilized or undeveloped eggs from developing larvae. Eggs hatch within about 96 h of fertilization (USEPA 1996; Thorpe et al. 2007). The resulting fry may be reproductively mature within 6 to 9 months, depending on rearing conditions.

The goal of the present study was to determine the nontarget effects of Zequanox on the survival and reproductive success of fathead minnows in simulated open-water applications. The specific objectives were to evaluate effects of 8-h exposure to 50 mg A.I./L and 100 mg A.I./L on: 1) survival of adult fathead minnows, 2) egg deposition, 3) egg hatchability, and 4) survival and growth of newly hatched fry.

Methods

Test System

Three separate tests were conducted in an outdoor mesocosm that consisted of 1000-L (total volume 980 L) circular tanks (high density polyethylene, 175 cm diameter x 64 cm height) located in two 0.004- hectare (ha) concrete ponds (Fig. 1). Daily and diurnal fluctuations in water temperature were minimized by filling the concrete ponds with well water to provide a thermal

jacket for the test tanks and by covering the ponds with black shade cloth. Pond water was pumped from a 0.1 ha earthen pond, passed through a 400 µm filter and delivered to a head-box system for distribution to nine test tanks per pond. Flow rate was adjusted to approximately 3.8 L/min per tank (~ 6 tank-exchanges/day). Aeration was supplied during the post-exposure rearing period through individual airstones in each tank that were connected to a regenerative blower. Aeration was not supplied during Zequanox exposure to simulate conditions in an open water application and assess treatment-related effects on dissolved oxygen concentrations.

Figure 1. Mesocosm test system consisting of nine 1000-L tanks within each of two 0.004 ha concrete ponds. Water was supplied to test tanks from a 0.25 ha earthen pond. Concrete ponds were filled with water and covered with shade cloth to moderate diurnal temperature fluctuation.

Zequanox Treatment and Concentration Verification

Two concentrations of Zequanox (50 and 100 mg Zequanox A.I./L) and an untreated control were tested at the expected environmental exposure duration (8h). The test material, Zequanox, was produced by Marrone Bio Innovations, Inc. (Davis, CA) and was a spray-dried powder formulation containing 50 % (w/w) active ingredient (*P. fluorescens,* strain CL145A). A dosing stock was prepared for each individual tank with water from the tank and the appropriate weight of dry Zequanox and added to the test tank within 5 minutes of preparation. Water flow to each tank was halted during the 8 h treatment. At the end of the exposure period, tanks were drained to half-volume and water flow was restored after the exposure.

The concentration of Zequanox (A.I.) in each test tank was determined by spectrophotometric comparison to a linear regression created from Zequanox standard solutions (Beckman UV/Vis Spectrophotometer, Model DU 800). Zequanox standards of 25, 50, 100, and 150 mg Zequanox A.I./L were prepared from serial dilutions of a 2,000 mg/L standard stock solution. A linear zero-intercept standard curve was prepared from the 25, 50, 100, and 150 mg Zequanox A.I.//L dilution stocks using triplicate standard samples. The spectrophotometer was blanked using filtered (200 µm) pond water. Mid-column water samples were collected from

each test tank for Zequanox concentration analysis at 0, 1, 2, 4, 6, and 8 h of exposure and at 1, 4, 8 and 16 h post-exposure. Confirmatory post-test efficacy verification of Zequanox was completed at MBI, Davis, CA. Results of post-efficacy assessment trials met the quality control standards set forth by MBI for the product.

Water Chemistry

Dissolved oxygen, temperature, and pH were measured daily in each tank during the pre- and post-exposure period and at 1 h and 8 h during the exposure period. Dissolved oxygen was measured with a YSI® 550A dissolved oxygen meter. The pH was determined with a Beckman Coulter® φ410 pH meter and probe. Temperature was measured with a Thermapen® digital thermometer. Water flow rates (mL/min) were measured daily in each tank. Total hardness (mg/L as $CaCO_3$) was determined by titrimetric method with Manver Red indicator (USEPA 1983). Total alkalinity (mg/L as $CaCO_3$) was determined by titrimetric method to a pH endpoint of 4.5 (APHA 1995). Conductivity was measured with a Fisher Accumet® conductivity meter. Hardness and alkalinity were measured from one replicate of each treatment before exposure and once during the exposure period. During the post-exposure period, hardness and alkalinity were measured weekly on the source water. Conductivity was measured in each tank before and once during the exposure period.

Adult Spawning Trial

Adult fathead minnows, 9 to 16 mo-old, were obtained in June 2014 from fish culture facilities at the Upper Midwest Environmental Sciences Center (UMESC). Fish were sedated with Aqui-S®20E (16 mg eugenol A.I./L), hand sorted by sex, and transferred into a partitioned raceway. Males were identified by the presence of tubercles on the head, a black spot on the dorsal fin and/or a dark band behind the head (Flickinger 1969). Females were identified by a lack of the aforementioned features and the appearance of the ovipositor (Flickinger 1969). Fish without defined sexual characteristics were omitted from the study. Forty female and 15 male fish were randomly distributed to nine test tanks (three replicate tanks per treatment). Mean total length of females was 62.8 mm (range 50.0-76.1 mm; SD=4.1) and of males was 70.1 mm (range 56.9- 85.0 mm; SD=5.3). Mean wet weight of females was 2.67 g (range 1.59-3.83 g; SD=0.48) and of males was 4.11 g (range 2.14-7.70 g; SD=0.89).

The following day, 10 spawning substrates were placed into each tank to monitor baseline egg production. Spawning substrates were constructed of a 15-cm length of 10-cm i.d. (inner diameter) polyvinyl chloride (PVC) pipe that was cut in half lengthwise (Fig. 2A). Substrates were observed daily for 5 days to verify that fish in each tank were in spawning condition and to provide an estimate of pre-exposure egg deposition. After the 5-d baseline spawning period, Zequanox was applied to the test tanks as described in the section *Zequanox treatment and concentration verification*. Water flow and aeration were halted to test tanks during treatment. Following Zequanox exposure, adult fish mortality and egg deposition were monitored daily for 21 days. Spawning substrates were removed from the tanks and examined; those with <50 eggs were cleaned and returned to the tank. All substrates with > 50 eggs were photographed for enumeration of eggs. The first ten substrates (1-10) with >50 eggs were transferred to a 1000-L rearing tank, corresponding to the adult treatment tank, in an adjacent concrete pond. When a substrate was removed from a test tank, a replacement substrate was placed into the tank to maintain a total of 10 substrates in the tank.

Spawning substrates 1-10 were incubated in the rearing tank in an upright position atop a mounted grill grate (57 cm diameter x 30 cm height) above a bubble wand (120-cm bubble wand) to maintain airflow over the eggs and reduce growth of fungus (Fig. 2B). Additionally, substrates were immersed in a fungicidal treatment (1667 mg/L formalin bath for 15 min) on three consecutive days (Schnick 1973). Substrates 1-10 were photographed again at 48 to 72 h to enumerate the number of eyed-eggs and assess fertilization and hatchability rates.

At the conclusion of the 21-day post-exposure spawning period, adult fish were euthanized in tricaine methanesulfonate (MS-222), sexed, measured and weighed. Unrecovered fish were counted as a mortality. The final count of females per tank (range =25 to 40, mean=33, SD=4) was used in analyses of egg production. Condition factor of adult fish was calculated as K = 100 (W/L^3) where K = condition, W = wet weight (g), L = total length (cm) (Nash et al. 2006). Cumulative egg deposition per female in a tank was enumerated over the 21-d post-exposure period. Percent eyed-eggs was determined for substrates 1-10 and was defined as: (number of eyed-eggs on substrate/number of eggs deposited on substrate) x 100.

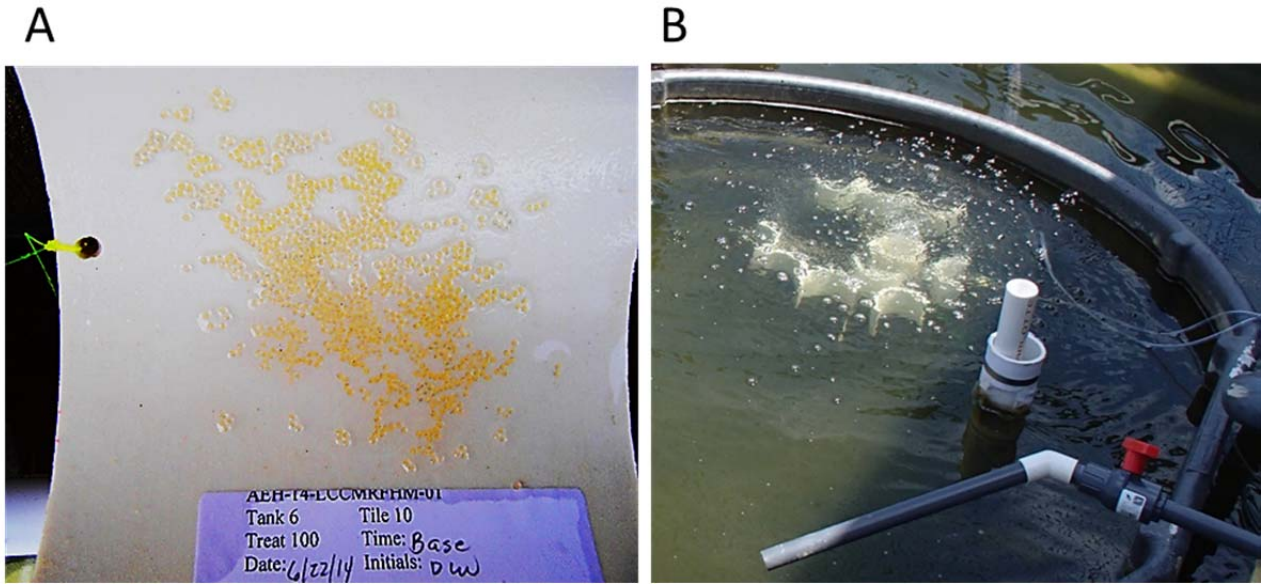

Figure 2. A) Spawning substrates with newly deposited eggs on the concave surface. A digital photograph was taken of substrates for enumeration of newly deposited eggs and eyed-eggs. B) Incubation of spawning substrates in the test tank atop a mounted bubble wand.

Egg Trial

Mature fathead minnows were transferred from indoor culture facilities into outdoor 0.04 ha concrete ponds in early June 2014. About 150-180 spawning substrates were placed into each pond and checked at about the same time each day for egg deposition. Substrates with >50 eggs (<24-h old) were placed into a cooler of pond water and immediately transferred to the mesocosm for distribution to test tanks. Ninety substrates were marked with an identification number and randomly distributed to one of nine test tanks (three replicate tanks per treatment) for a total of 10 substrates per tank. Within 4 h of initial substrate collection from the pond, Zequanox was applied to the test tanks (as described in the section *Zequanox Treatment and Concentration Verification).* At 1-h post-exposure, all substrates in a tank were removed and simultaneously immersed in a formalin bath, as described in the section *Adult Spawning Trial,* returned to the tank, and incubated on a grill grate with aeration (Fig. 2B). Substrates were

individually photographed on Day 0 and Day 3 for enumeration of initial egg deposition and number of eyed-eggs, respectively. Percent hatchability was defined as: (number of eyed-eggs on substrate/number of eggs deposited on substrate) x 100.

Fry from the adult spawning and egg trials were maintained in separate replicate treatment tanks and reared in the outdoor mesocosm until October 2014. Estimates of fry mortality were confounded by the occurrence of several disease outbreaks and potential nutritional deficiencies; therefore, data collection was terminated at 30 d post-exposure.

Fry Trial

Adult fathead minnows were transferred from indoor culture facilities at UMESC to two outdoor ponds in early June 2015. Spawning substrates were placed throughout the ponds and checked at the same time each day for newly deposited eggs. When sufficient eggs were found deposited on one day, substrates were placed into a cooler of pond water and transferred to a wet lab. Eggs were stripped from substrates by immersion in a de-adhesion solution of sodium sulfite (15 g/L) for approximately 3 minutes followed by immersion in a bucket of well water and collection of sloughed eggs. Eggs were transferred to a McDonald upwelling incubator jar for hatching. Water temperature was maintained at 23°C and flow rate was ~7.5L/min. Newly hatched fry (<24-h old) were collected on Day 4 of incubation and randomly distributed (n=300 fry per replicate) to each of 18 test tanks (6 replicates per treatment) in the outdoor mesocosm.

To observe fry during the exposure, a 100-L stainless steel vessel (35 cm x 60 cm x 60 cm, H x L x W) was used to hold fry during the exposure period. Fry were allowed to acclimate overnight and the following day, Zequanox was applied to the stainless steel vessel (as described in the section *Zequanox Treatment and Concentration Verification)*. At the end of the 8-h exposure, the contents of the stainless steel vessel were poured into the 1000-L tank; water flow was established and air stones were placed into each test tank. Daily observations of fry mortality were recorded in each tank for 90 days.

On days 44 and 90 post-exposure, condition factor was determined from a subsample of 20 fish per tank. Fish were euthanized in MS-222 (250 mg/L) and then weighed (wet weight, 0.01 g) and measured (total length, 0.1 mm). Total fish survival was determined on day 90. Each test tank was drained and fish were transferred to a 9.5 L bucket for euthanization in MS-222. The total number of fish was counted and a total wet weight was obtained for each test tank.

Data Analysis

In every analysis, the tank was treated as the experimental unit. All statistical analyses were performed using SAS Version 9.3 (SAS Institute, Inc.) and statistical significance was defined at $\alpha < 0.05$. Analysis of water chemistry (dissolved oxygen, pH, temperature, alkalinity, water hardness, and conductivity) and exposure concentration were summarized with simple descriptive statistics. Egg production per female (adult trial) was compared across treatments with Kruskal-Wallis nonparametric test (Proc nparway1). Percent eyed-eggs (adult and egg trial) and percent mortality at 90 days (fry trial) were analyzed with a generalized linear mixed model (Proc glimmix) with treatment as a fixed effect, tank as a random effect, and a binomial logistic regression (logit link function) with random intercepts. A scale parameter was added to the model using the random_residual_statement. Responses of each treatment group were individually compared to the control group using a two-sided least squares means (LSD) comparison test. Condition factor of adult fish (21 d post-exposure) and fry (44 and 90 d post-exposure) was modeled using a mixed effects model (Proc mixed) with treatment as a fixed

effect and tank as a random effect. Condition factor (by sex) of the control groups was compared to that of the treatment groups using a two-sided LSD comparison test.

Results

Water Quality and Zequanox Concentration

Mean measurements of dissolved oxygen, pH, and temperature were similar for the three trials (Table 1). Mean temperature ranged from 24.9°C to 26.2°C during the exposure and from 21.5°C to 22.3°C during post-exposure period of the three trials. A diurnal fluctuation in pH occurred in all tanks in the three trials that ranged from a morning low of about 8.10 to a peak of about 9.60 at late afternoon (Table 1). Dissolved oxygen concentrations decreased slightly during the 8-h exposure in the treatment tanks compared to the control tanks, but remained >7.9 mg/L in all tanks (Table 1).

The mean concentration of Zequanox was similar among replicates in the three trials (Table 2). Overall, the lowest mean concentrations occurred in the adult spawning trial and the highest occurred in the fry trial (Table 2). Differences in measured concentrations of Zequanox among trials are partly attributed differences in the volume of the test tanks between the adult/egg trials and fry trials and settling of the product during the 8-h exposure period.

Adult Spawning Trial

Baseline spawning (pre-exposure) occurred in every tank. The number of spawning substrates with eggs deposited ranged from 4 to 13 per tank and the total number of eggs ranged from 2156 to 8974 per tank. Mean number of eggs per substrate was 700, SE=163.
A spawning event occurred on every day of the 21-d post-exposure spawning period in at least one tank and spawning occurred consistently throughout the 21-d period, except for a slight downward trend in the last 3 days of the trial (Fig. 3). The total number of spawned substrates ranged from 18–32 per tank and was not related to treatment (chi-square=0.81, p=0.67, 2 df). Mean cumulative egg production per female ranged from 344 eggs, SE=77 (50 mg/L Zequanox) to 409 eggs, SE=49 (control) and did not differ significantly among treatments (chi-square=1.01, p=0.59, 2 df) (Fig. 4). Additionally, there was no significant effect of Zequanox treatment on fertilization and development to the eyed-stage of fathead minnow eggs on the first ten spawning substrates (p=0.11, F=2.26, 2 df) (Fig. 5). Although there was a trend downward in percent eyed-eggs in the 100 mg/L treatment, differences among treatments were not significant (p=0.11, F=2.26, 2 df).

Total mortality of adult fish at 21-d post exposure ranged from 0% to 12% (n=0 to 7 fish per tank). Mean mortality in the three treatments was 4% (controls), 3% (50 mg/L) and 6% (100 mg/L). Mean condition factor of fish at 21-d post-exposure did not differ (within sex) between control and treatments (females, p=0.93, F=0.93; males p=0.262, F=1.33, 2 df). Mean condition factor of females was 1.07, standard error (SE) =0.02 (control), 1.07, SE=0.01 (50 mg/L) and 1.08, SE=0.02 (100 mg/L). Mean condition factor of males was 1.15, SE=0.01 (control), 1.18, SE=0.02 (50 mg/L) and 1.22, SE=0.03(100 mg/L).

Table 1. Mean (SD) dissolved oxygen (DO) and temperature and pH range during Zequanox trials of fathead minnow adults, eggs, and newly hatched fry.

Water quality parameter	Treatment group	Adult trial	Egg trial	Fry trial
		Exposure		
DO (mg/L)	Control	9.32 (2.19)	9.23 (1.35)	9.02 (0.63)
	50 mg/L	8.13 (0.49)	8.84 (1.74)	8.74 (0.57)
	100 mg/L	7.91 (0.63)	8.56 (1.85)	8.71 (0.53)
pH range	Control	8.94 (8.85–9.10)	8.86 (8.54–9.22)	8.75 (8.39–9.26)
	50 mg/L	8.77 (8.64–8.88)	8.75 (8.57–8.93)	8.63 (8.31–9.25)
	100 mg/L	8.63 (8.33–8.87)	8.59 (8.55–8.64)	8.60 (8.02–9.25)
Temp (°C)	Control	26.2 (1.65)	26.0 (1.05)	24.9 (1.80)
	50 mg/L	26.3 (1.66)	26.0 (1.10)	24.7 (1.72)
	100 mg/L	26.3 (1.72)	26.1 (1.04)	24.9 (1.79)
		Post-exposure observation		
DO (mg/L)	Control	8.86 (2.20)	9.27 (1.03)	8.13 (0.87)
	50 mg/L	8.86 (2.22)	9.27 (0.98)	8.11 (0.88)
	100 mg/L	8.87 (2.13)	9.20 (0.97)	8.14 (0.89)
pH range	Control	8.78 (8.13–9.62)	8.89 (8.31–9.94)	8.53 (7.77–9.57)
	50 mg/L	8.79 (8.14–9.61)	8.90 (8.31–9.94)	8.55 (8.04–9.57)
	100 mg/L	8.78 (8.13–9.60)	8.89 (8.33–9.92)	8.53 (8.02–9.63)
Temp (°C)	Control	22.3 (1.48)	21.5 (1.56)	21.7 (2.37)
	50 mg/L	22.3 (1.49)	21.5 (1.57)	21.7 (2.37)
	100 mg/L	22.3 (1.47)	21.5 (1.56)	21.6 (2.36)

Table 2. Mean concentration (SD) of Zequanox during 8-h exposure period in three replicate tanks.

Treatment group	Adult	Egg	Fry
Control	ND[1]	ND	ND
50 mg/L	47.3 (4.2)	49.6 (5.6)	54.7 (2.2)
100 mg/L	87.7 (6.4)	93.9 (8.9)	100.7 (6.9)

[1]Not detected.

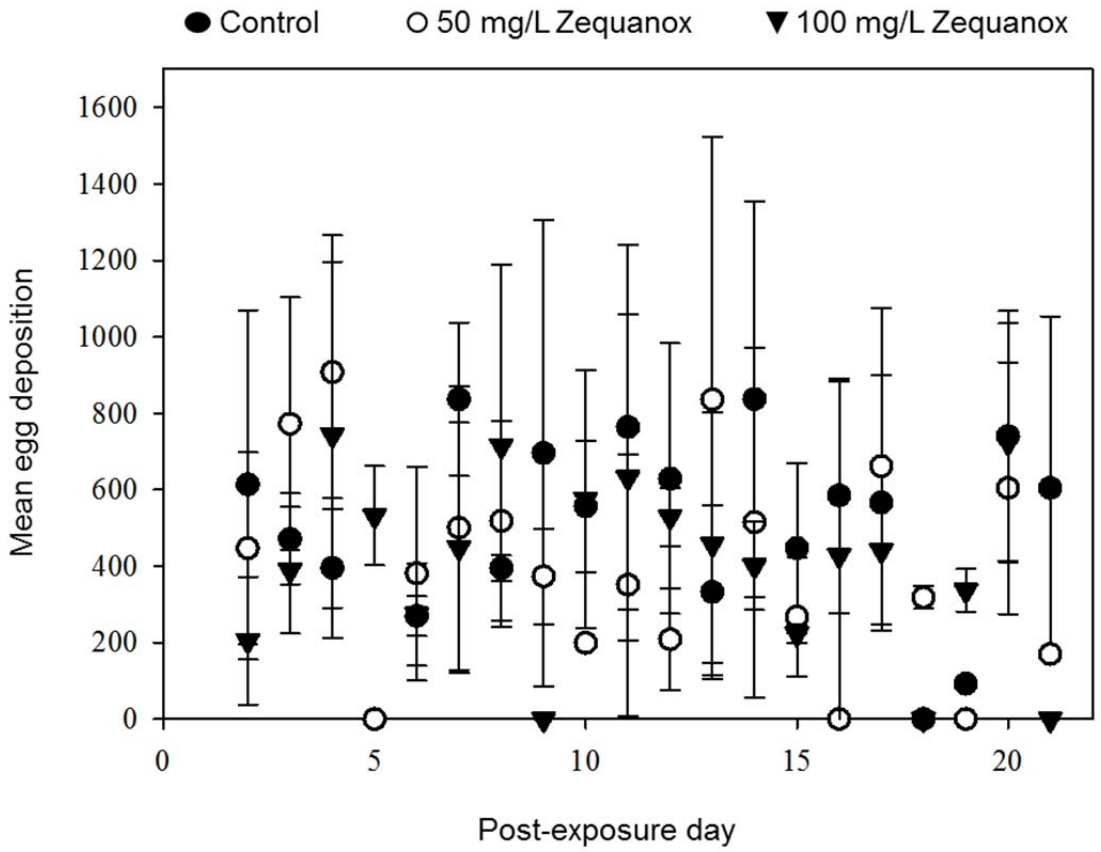

Figure 3. Adult trial: Mean (standard error, SE) daily egg production per female fathead minnow after Zequanox treatment.

Egg Trial

Zequanox treatment did not have a significant effect on development to the eyed-stage of deposited eggs (Fig.6). The percent of eggs that developed to the eyed-stage ranged from 92.6%, SE=0.02% (50 mg/L) to 94.8 %, SE=0.01% (control) and was not significantly different between the control and treatments (p=0.82, F=0.82, 2 df).

Fry Trial

Zequanox treatment did not have a significant effect on 90-d survival of newly hatched fathead minnow fry. Cumulative mean survival ranged from 83.7%, SE=0.02% (100 mg/L) to 81.4%, SE=0.01% (50 mg/L) (Fig. 7) and did not differ significantly between control and treatments (p=0.54, F=0.64, 2 df). Additionally, the 44-d (p=0.75, F=0.29, 2 df) and 90-d (p=0.30 f=1.21, 2 df) condition factor of fry was not significantly different among control and treatments (Fig. 8).

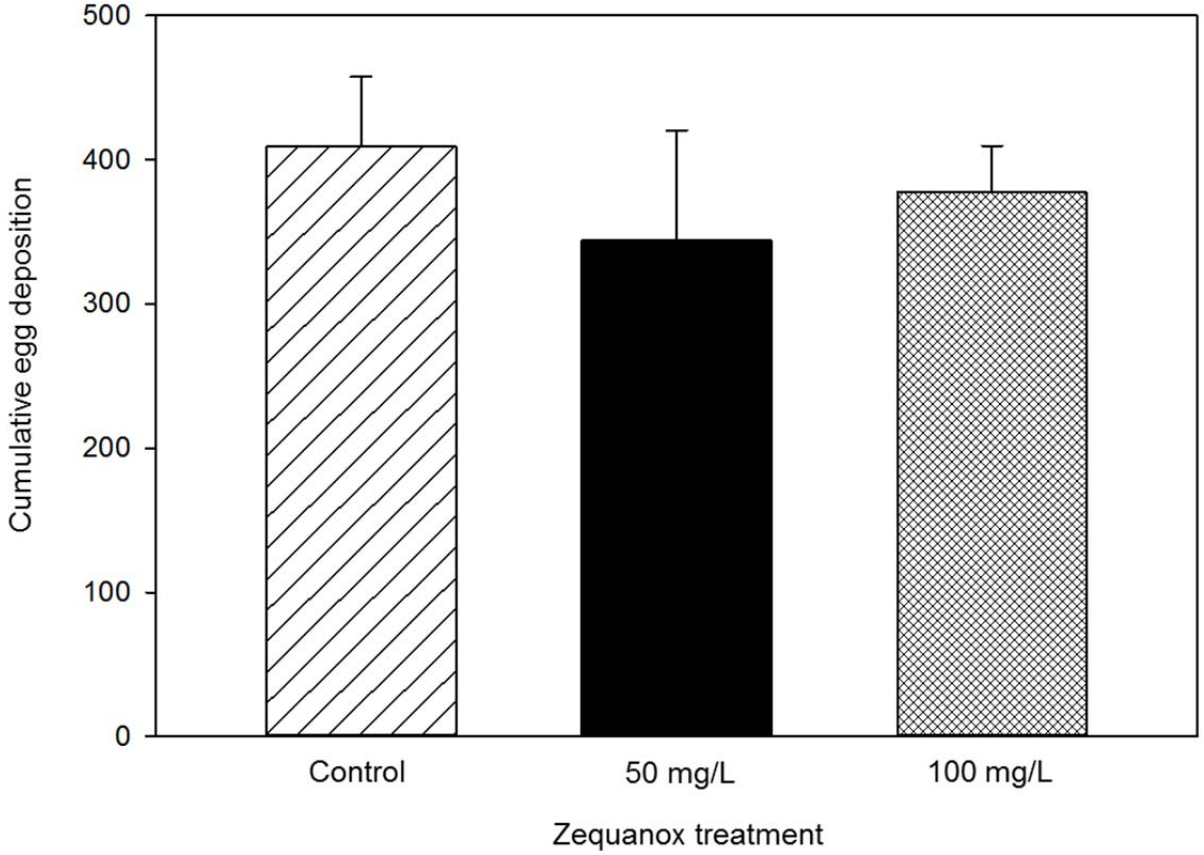

Figure 4. Adult trial: Mean (SE) 21-d cumulative egg deposition per female after Zequanox treatment.

Conclusions and Discussion

Our results indicate that Zequanox treatment at the maximum approved concentration and exposure duration is safe for adult and early life stages of fathead minnow. Mortality of adults and newly hatched fry was minimal in all treatments. The mode of action of Zequanox is through ingestion and degradation of the digestive epithelium (Molloy et al. 2013c). As a result, organisms that are susceptible to the toxic component in Zequanox may have reduced growth and condition rather than overt mortality. Luoma et al. (2015) reported reduced condition factor at 22 d post-exposure in several fish species for which the LC50 value was >100 mg/L. For example, the 24-h LC50 value for largemouth bass was 173.6 mg/L, but condition factor was significantly less in fish exposed to ≥75 mg/L Zequanox. In the present study, mean condition factors of adults and newly hatched fry were not significantly different among fish in the control and Zequanox treatments (Fig. 8).

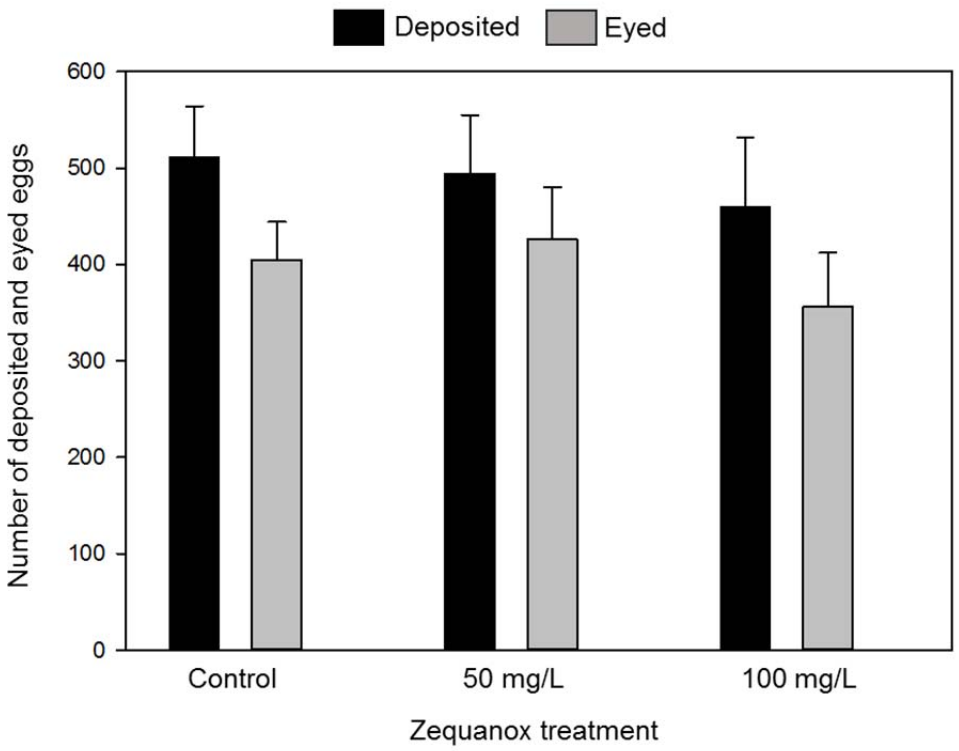

Figure 5. Adult trial: Mean (SE) egg deposition and development to eyed stage (Substrates 1-10) of fathead minnow eggs after Zequanox treatment.

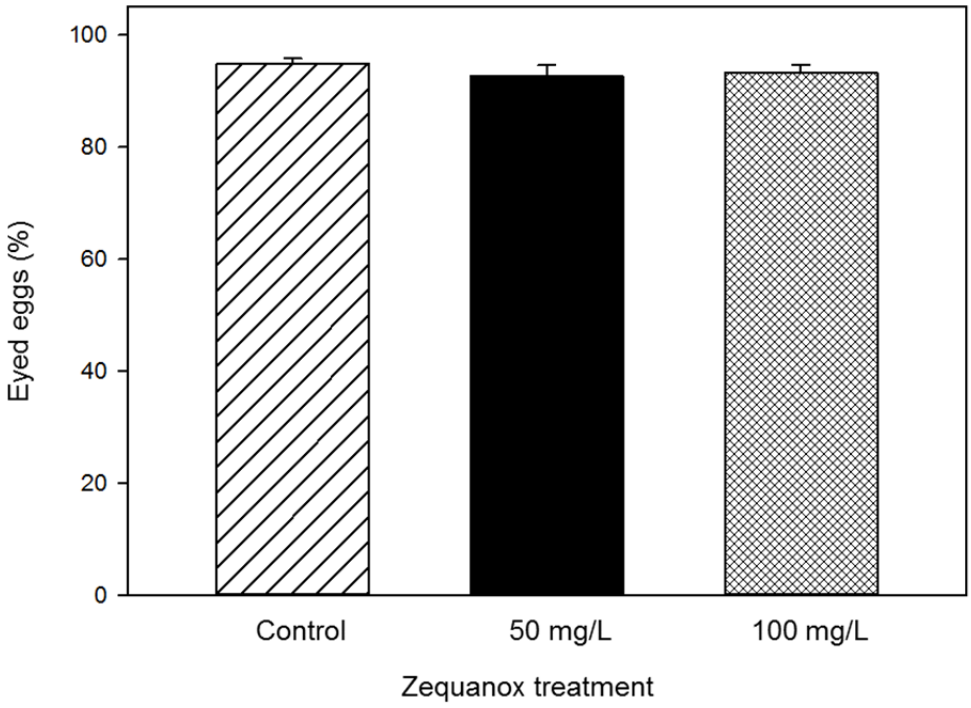

Figure 6. Egg trial: Mean percentage (SE) of fathead minnow eggs developing to eyed-stage after Zequanox treatment.

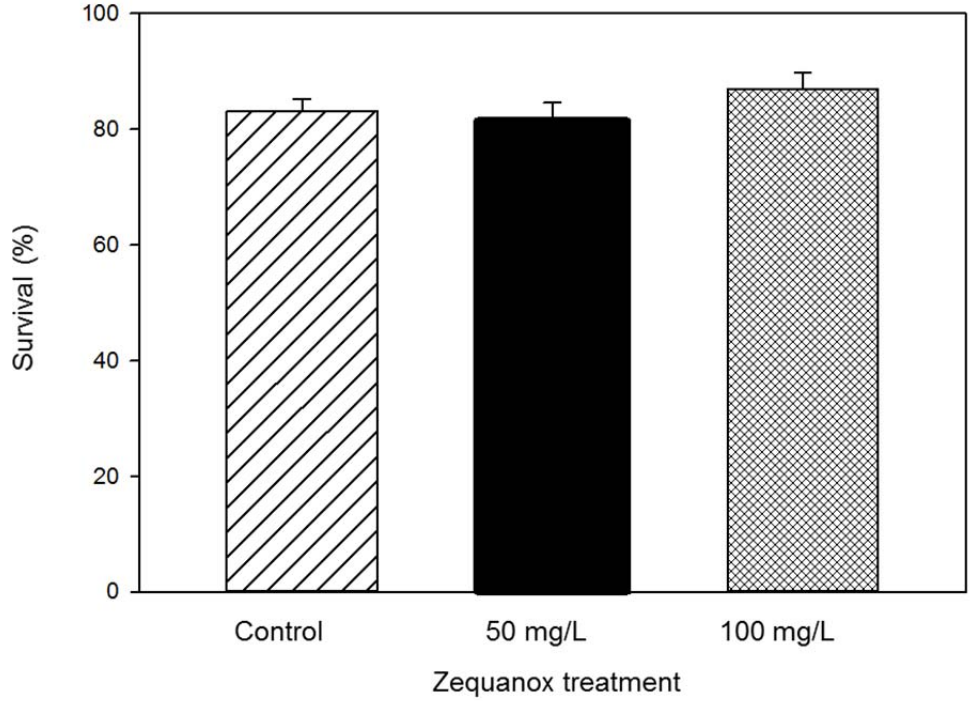

Figure 7. Fry trial: Mean percent survival (SE), 90-d post-exposure, of fathead minnow fry after Zequanox treatment.

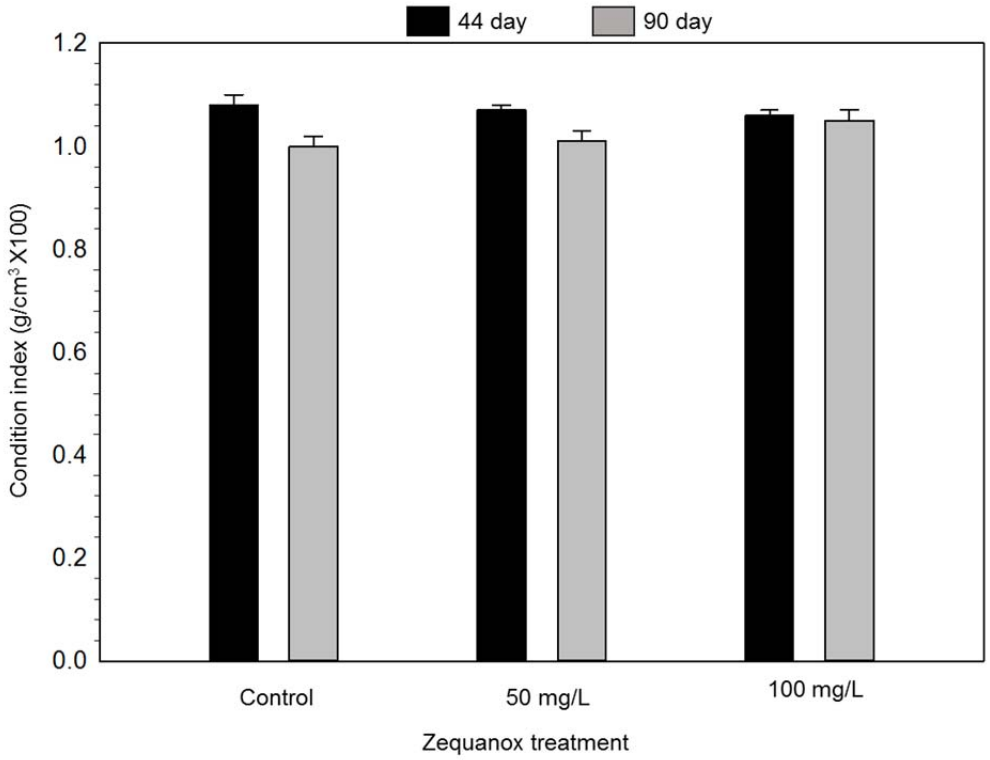

Figure 8. Fry trial: Mean (SE) condition factor of fathead minnow fry after Zequanox treatment.

By all measures used in the present study, Zequanox had no negative effect on spawning of fathead minnows during or after exposure (Figs. 3 and 4). Zequanox is composed primarily of organic particulates and produces a highly turbid suspension in the water column. Eggs were found on spawning substrates on Day 1 post-exposure indicating that turbidity did not prevent spawning or fertilization during the 8-h exposure (Fig. 3). There was no direct or lingering effect of Zequanox on egg deposition and fry development at the concentrations and exposure duration that were tested (Figs. 3-8). Females continued to deposit eggs during the post-exposure period and development of those eggs to the eyed-stage was similar in all treatments (Fig. 5).

The results suggest several potential applications for Zequanox in fishery management. The biopesticide may be used for removing dreissenid mussels from fish spawning shoals and reefs as part of a dreissenid integrated pest management program. Zequanox may be one alternative for killing dreissenid veligers and settlers in waters that contain fish or eggs, such as aquaculture ponds, when KCl-formalin combination or other salts are ineffective or unsafe for the species or life stage of fish.

However, the use of Zequanox in waters that contain fish will depend on the fish species. Some cool-water fish species may be more sensitive to Zequanox than fathead minnows and other warm-water species. For example, the survival and condition factors of fingerling lake trout (J. Luoma, personal communication) and rainbow trout (Luoma et al. 2015) were reduced after Zequanox treatment. Before large scale treatment of fish with Zequanox is conducted, trial exposures with the species and life stage of concern are recommended to verify its safety.

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