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Preliminary Test of an Electrical Barrier to Prevent the Upstream Movement of New Zealand Mud Snails

INSIDE THIS ISSUE:

Introduction

During the past century, several hundred exotic aquatic plant and animal species have been introduced into North America (Mills et al. 1993; Ricciardi and Rasmussen 1998). Many of these species were inadvertently spread through aquacultural practices (Naylor et al. 2001). While not originally documented in an aquaculture facility, New Zealand mud snails (*Potamopyrgus antipodarum*; syn. *Hydrobia jenkinsi*; NZMS) are an aquatic nuisance species that are now found in many locations in the western United States (Bowler 1990). Because they can consume a significant portion of stream gross primary production (Hall et al. 2003) and may account for the majority of invertebrate production (Hall et al. 2006), NZMS frequently out-compete other invertebrates both numerically and through exploitative competition (Hall et al. 2006). Because of the potential effect they have on wild fish and invertebrate populations, actions should be taken to prevent the spread of NZMS. Since NZMS can survive passage through the digestive tract of rainbow trout *Oncorhynchus mykiss* (Bruce 2006), stocking of fish from NZMS infested hatcheries could lead to inadvertent spread of the species.

In November 2007, NZMS were identified in the raceways of the Utah Division of Wildlife Resources' (UDWR) Loa State Fish Hatchery. Several other UDWR hatchery facilities are located upstream of wild NZMS populations. UDWR officials are concerned that NZMS could move upstream into these other facilities. In this article, we present results from preliminary research conducted by the UDWR's Fisheries Experiment Station to develop an electrical barrier that can be installed at other hatchery facilities to prevent the upstream movement of NZMS.

Biologist Randy Oplinger places New Zealand Mud Snails in raceway troughs at Loa hatchery in a previous experiment

Methods

To test the effect of electricity on NZMS behavior, four artificial streams were created. Each 'stream' consisted of two parallel, 150 cm long sections of housing gutter. The two gutter sections ended in a common collection basin that was also constructed from housing gutter. A 2.5 cm wide copper strip was glued 30 cm from the downstream end of each parallel gutter. A second copper strip was installed 2.5 – 10.0 cm upstream of the first strip. These copper strips served as electrodes and the separation between the strips was selected in such a manner than when 24.0 v of direct current (DC) was applied to the electrodes, four electrical current densities were created (0.7, 1.5, 3.4, and 4.2 mA/in^2). The artificial streams were placed into raceways at the Loa Hatchery and siphons were started to provide water for each stream. A DC power supply (BK Precision Model 1715, set at 24.0 V) was used to electrify the copper strips in one gutter section of each artificial stream. The other side was not electrified and served as a control.

(Continued on page 2)

(Continued from page 1)

One hundred NZMS were placed into the collection basin of each artificial stream. After 24 h, the number of snails in the electrified and non-electrified gutters in each artificial stream was counted. In addition, the number of snails between and upstream of the copper strips (on both sides) was counted. Snails were then removed from the gutters and a new batch of 100 NZMS were added to each collection basin. A total of four replicate 24 h runs were performed in each artificial stream.

Table 1: Average (SE in parentheses) number of snails that crossed the two copper strip electrodes on the electrified and control (non-electrified) sides of artificial streams that produced four different electrical current densities.

Electrical Current Density (mA/in^2)	Electrified	Control
0.7	1.25 (0.9)	2.25 (1.1)
1.5	0.75 (0.5)	4.0 (1.6)
3.4	3.75 (1.3)	6.25 (2.2)
4.2	0.0 (0.0)	2.25 (0.9)

Results and Discussion

Overall, we found that the presence of the non-electrified copper strips had no effect on NZMS behavior (Table 1). We found that on average, 7.5 ± 1.3 (mean ± SE) NZMS entered the control (non-electrified) gutter of each artificial stream. Of these NZMS, on average 3.7 ± 0.8 NZMS successfully crossed the copper strips. We observed large variability in the number of snails that crossed the electrodes on the electrified side of each artificial stream. Therefore, the number of snails that crossed the two electrodes did not differ significantly among electrical current densities (ANOVA, $F_{3,12} = 1.46$, $P = 0.27$). On average, 1.2 ± 0.5 NZMS crossed the two electrodes in the electrified gutter of each stream. Regardless, through four replicate, 24 h runs, we observed that no NZMS crossed the electrodes in the artificial stream with the highest electrical current density (4.2 mA/in^2).

These results suggest that electrical current densities ≥ 4.2 mA/in^2 could potentially be used to prevent the upstream movement of NZMS into hatchery facilities. While encouraging, these results are preliminary. Researchers at FES are working on designing a raceway scale electrical barrier that they will construct and test at Loa. The results of this large-scale barrier will be used to make recommendations on the design of electrical barriers that can be constructed at other UDWR hatchery facilities to prevent the upstream movement and colonization by NZMS.

Randy Oplinger

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Rainbow Trout Egg Disinfection with Higher Doses of Hydrogen Peroxide and Iodine for Shorter Durations

Egg disinfection plays a critical role in improving the survival to hatch for many fish species reared in captivity. Both fungal and bacterial agents have been implicated in reduced survival of fish eggs (Gee and Sarles 1942; Burrows 1949; Ross and Smith 1972; Barker et al. 1989; Barnes et al. 2003). Compounds to control these pathogens have been limited, despite screening of numerous chemical candidates (Bailey and Jeffrey 1989; Marking et al. 1994). Iodine at 100 mg/L for 10-15 min has been the standard egg treatment for many years based on work by McFadden (1969) and Amend (1974), but recent data has suggested that an alternative is necessary for adequate disinfection (Kumagai et al. 1998; Wagner et al. 2008).

The objective of the egg disinfection research summarized in this article was to explore the potential of higher concentrations of iodine and hydrogen peroxide applied for shorter durations.

Eyed rainbow trout eggs were treated with iodine or hydrogen peroxide. Treatments were (1) 100 mg/L iodine for 15 min (control), (2) 2,000 mg/L iodine for 10 min, (3) 0.6% hydrogen peroxide for 5 min, (4) 3.0% hydrogen peroxide for 1 min, and (5) untreated eggs (no chemical treatment, but similarly handled). Survival rates were slightly, but significantly, lower for eggs treated with iodine (Table 1). However, hatching rates were above 93% for all treatments. Deformity rates were less than 1% for all treatments, and did not significantly differ. The survival and deformity data indicated that there were no ill effects of the higher chemical concentrations used in the study.

Table 1. Mean (n = 3) percent survival to hatch and the percentage of alevin deformities among various chemical treatment concentrations and durations of iodine and hydrogen peroxide. Means followed by a common letter within a column are not significantly different (P > 0.05); controls had only one replicate, so were not included in the statistical analysis.

Treatment	Duration (min)	Hatch (%)	Deformity rate (%)
0.6% hydrogen peroxide	5	95.7 a	0.15 a
3.0% hydrogen peroxide	1	95.5 a	0.50 a
2,000 mg/L iodine	10	94.0 b	0.47 a
100 mg/L iodine	15	93.7 b	0.35 a
Control		96.1	0.10

Counts of bacterial colony forming units (CFU; Table 2) indicated that chemical treatment with either iodine or hydrogen peroxide significantly reduced bacterial abundance on the rainbow trout eggs relative to untreated eggs, but some bacteria still persisted on the eggs. The 3.0% hydrogen peroxide for 1 min treatment was significantly better than all the other treatments in 2 of 3 replicates for egg samples plated on EOT media and in 1 of 3 replicates for eggs on TSA media (Table 3). Differences in CFU counts between 2,000 mg/L iodine for 10 min and 0.6% hydrogen peroxide for 5 min were few and variable. For iodine treatments, there were significantly fewer CFUs in the 2,000 mg/L iodine treatment in 4 of the 6 replicate groups than in the 100 mg/L treatment (Table 3).

We had a total of eleven bacteria isolates representing the diversity of bacteria that grew on agar from the eggs in the study. All the isolates were Gram-negative and all but one were rod shaped. Further identification was attempted with the API system, but was inconclusive.

(Continued on page 4)

Table 2. Frequency distribution of extrapolated CFU counts on two different media (TSA and EOT) for eggs treated with various chemical treatment concentrations (ppm) and durations of iodine (I) and hydrogen peroxide (H).

			EOT				TSA			
Treatment	min	Rep	0	1-300	300-1,000	>1,000	0	1-300	300-1,000	>1,000
I 100	15	1	3	2	3	2	5	3	0	2
		2	0	0	0	10	0	4	5	1
		3	0	2	5	3	0	8	1	1
I 2,000	10	1	4	2	2	2	9	8	1	0
		2	0	0	0	10	0	7	1	1
		3	0	0	1	1	1	0	0	1
H 6,000	5	1	0	5	5	0	0	10	0	0
		2	0	0	0	10	0	6	2	4
		3	0	3	2	5	0	7	1	1
H 30,000	1	1	0	9	1	0	3	6	1	0
		2	0	5	5	0	2	8	0	0
		3	6	4	0	0	7	2	1	0
Untreated		1	0	0	0	10	0	0	0	10
		2	0	0	0	10	1	0	0	9
		3	1	0	0	9	0	0	0	10

Table 3. Mean ranks of CFU counts for each replicate and media type for rainbow trout eggs disinfected with various concentrations (mg/L) and durations of iodine (I) or hydrogen peroxide (H). Means followed by a common letter within a column are not significantly different ($P > 0.05$).

Treatment	Duration (min)	EOT			TSA		
		Rep 1	Rep 2	Rep 3	Rep 1	Rep 2	Rep 3
I 100	15	21.8 bc	28.0 b	28.9 b	19.9 c	31.0 a	25.5 b
I 2,000	10	19.2 bc	28.0 b	18.7 c	11.1 d	18.2 bc	18.5 c
H 6,000	5	45.4 a	42.8 a	41.8 a	45.3 a	36.9 a	45.3 a
H 30,000	1	25.4 b	23.2 c	31.5 b	31.5 b	28.7 ab	28.0 b
Control	0	15.6 c	5.5 d	6.6 c	19.7 c	12.6 c	10.1 d

The 3.0% hydrogen peroxide and 2,000 mg/L iodine treatments both significantly reduced bacterial abundance without compromising egg survival and are recommended as a replacement for the current practice of 100 mg/L iodine for 10 to 15 min. Of the two options, hydrogen peroxide is probably cheaper and environmentally more benign. With iodine, toxicity is dependent on pH and stage of egg development (Amend 1974). So, the 2,000 mg/L recommended iodine dose could be lethal at pH 6.9 (LC_{50} = 1480 mg/L in 15 min treatment; Amend 1974), but safe at pH 7.0 or 8.0 (Alderman 1984). The toxicity of hydrogen peroxide to eggs varies among species (Rach et al. 1998: Gaikowski et al. 1999). For rainbow trout eggs in a 15 min treatment, mortality increased 1.4 to 5.9% at 500 ppm hydrogen peroxide, but could be increased to 1,000 ppm by avoiding prophylactic treatment during critical developmental stages (Gaikowski et al. 1998; Arndt et al. 2001). LC_{50} values for rainbow trout fry and fingerlings vary from 393 to 8,660 ppm depending on life-stage and temperature (Rach et al. 1997). Higher concentrations at shorter durations (3% hydrogen peroxide for 5 min) have been tested successfully on red drum *Sciaenops ocellatus* eggs, Atlantic cod *Gadus morhua,* and haddock *Melanogrammus aeglefinus* (Douillet and Holt 1994; Peck et al. 2004).

The 3% hydrogen peroxide egg disinfection procedure was implemented this year on a trial basis during the egg taking operations at the wild fish traps. Problems with poor survival to eye-up and hatch occurred in several

(Continued on page 5)

instances. As a result we conducted some further tests, measuring the effect of hydrogen peroxide upon pH at various hardness levels. The results indicated that as total hardness dropped, the greater the change in pH towards the acid range (Table 4). The pH dropped to levels that could be deleterious to fish eggs (Table 4). Given that hydrogen peroxide is denser than water (1.4067 specific gravity), problems may have also occurred when mixing the concentrated solution (35%) in with the eggs. The dense concentrate would sink to where the eggs are, where it may not be thoroughly mixed and induce mortality. So, for use of hydrogen peroxide we recommend mixing it beforehand and dipping the eggs in the solution, rather than pouring concentrated H_20_2 in the container with the eggs. Buffering for the pH effect is also recommended. Further testing of buffers is needed, as well as the effect of species, strain, and egg quality on survival. Further exploration of the 2 to 15 min range at the same or lower dose is also needed to better define the risks and benefits of various concentrations of hydrogen peroxide for rainbow trout eggs at all stages of development.

Table 4. Effect of hydrogen peroxide on pH at various levels of total hardness.

Total hardness (ppm)	pH without H_20_2	pH with H_20_2	delta pH
0	5.77	3.33	2.44
10	6.96	5.12	1.84
154	6.91	5.46	1.45
291	7.09	6.52	0.57

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Lethal Tests of Argentyne, Formalin, Hydrogen Peroxide, and Potassium Permanganate on New Zealand Mud Snails

Introduction

New Zealand mud snails (*Potamopyrgus antipodarum*; syn. *Hydrobia jenkinsi*; NZMS) are an aquatic nuisance species that were first reported in the western United States in 1987 (Bowler 1990). NZMS are herbivores and graze on benthic algae and macrophytes. They can be responsible for consuming up to 75% of the gross primary production (Hall et al. 2003) and may account for up to 92% of the invertebrate production in a stream (Hall et al. 2006). The fact that NZMS account for the majority of secondary production in a stream suggests that once established, NZMS may out-compete other invertebrates and become a dominant member of the invertebrate community. They may also exert an indirect bottom-up effect on fish populations.

The Utah Division of Wildlife Resources (UDWR) frequently collects eggs from wild fish for rearing in State hatcheries. Officials, however, are concerned that NZMS could be inadvertently collected during these operations and transported into the hatchery system. The purpose of this article is to present results of research conducted by the UDWR's Fisheries Experiment Station to identify chemicals that could be used to kill NZMS that are inadvertently collected during wild egg take operations. These same chemicals could have other applications in hatcheries to kill NZMS.

Methods

Solutions of Argentyne (0 [control], 50, 100, 1,000, 5,000, and 10,000 ppm [100% stock solution] free iodine), formalin (0 [control], 1,667, 16,667, 30,000, 60,000, 100,000, and 166,700 ppm), hydrogen peroxide (0 [control], 100, 250, 500, 1,000, 1,500, 2,500, 5,000, 7,500, 10,000, and 30,000 ppm), and potassium permanganate (0 [control], 1, 10, 100, 1,000, 10,000, 25,000, 50,000 and 100,000 ppm) were prepared in the laboratory and transported to the Loa State Fish Hatchery. The UDWR frequently treats eggs with either a 100 ppm Argentyne or 1,667 ppm formalin solution. The higher concentrations tested are not likely egg safe but were included so the toxicity of each chemical to NZMS could be determined. All NZMS used in this experiment were collected from raceways at the Loa State Fish Hatchery using a dip net (mean TL: 1.82 ± 0.05 mm, mean ± SE, $N = 50$). Groups of 25 NZMS were then counted into dry, 400 mL beakers. After counting, 200 mL of prepared chemical was poured into the beaker. After 15 minutes of exposure, the chemical was poured out of the beaker and the snails were rinsed three times with hatchery water. After rinsing, the beaker with NZMS was re-filled with 200 mL of water and held for 24 h. After the 24 h holding period the number of live snails was determined. At least four replicate groups of 25 snails were exposed to each concentration of each chemical. However, up to 12 replicate groups of 25 snails were exposed to some concentrations. In addition to 15 minute chemical exposures, we tested a 30,000 ppm hydrogen peroxide solution for timed exposures of 1, 10, 15, 25, 50, and 100 minutes. In this timed exposure, NZMS were held for 15 minutes in hatchery water as a control. Similar to the 15-minute treatments, NZMS were rinsed three times after exposure and held for 24 h to assess survival.

A one-way ANOVA was performed using the Proc MIXED procedure in SAS (SAS 1998) to detect survival differences among concentrations of each chemical. Differences were considered statistically significant at $P < 0.05$. Arcsine-square root transformations were utilized to ensure the data was normally distributed. Also, a probit analysis was performed using the Proc Probit procedure in SAS (SAS 1998) to determine LD_{50} and LD_{90} concentrations for each chemical.

Results and Discussion

NZMS survival varied significantly among the concentrations of each chemical tested (all $P < 0.01$). Of the therapeutic chemicals tested, potassium permanganate required the lowest concentration to produce significant mortality (Table 1). Interestingly, as potassium permanganate concentration increased from 0 to 100 ppm, survival dropped from 100% to 5.0 ± 1.46% (Figure 1). Then, survival increased at concentrations between 500

and 7,500 ppm. Survival then decreased to 3.0 ± 1.64% at 10,000 ppm and 0% at 100,000 ppm. In contrast, survival of NZMS treated with every other chemical decreased with each increase in concentration (Figure 1). Of the chemicals tested in the 15-minute trials, hydrogen peroxide required the lowest concentration for complete mortality (7,500 ppm; Table 1). In the timed trials with the 30,000 ppm hydrogen peroxide solution, 3.0 ± 1.91% survived a 1-minute treatment; however, 15 minutes was the shortest time interval tested where 100% of the snails died (Table 1). Estimated LD_{50} and LD_{90} values in the timed 30,000 ppm trials were less than 1-minute (Table 1). Because of it's high toxicity, we feel that hydrogen peroxide may be the most effective of the chemicals tested.

Table 1: Mean formalin, hydrogen peroxide, and free iodine New Zealand mud snail LD_{50} and LD_{90} values. 95% confidence limits are given in parentheses. Minimum tested at 100% mortality refers to the lowest concentration we tested that produced no survival. With the exception of the timed exposure experiment with hydrogen peroxide (data in seconds), all values are ppm.

Chemical	LD_{50}	LD_{90}	Minimum Tested at 100% Mortality
Iodine	187 (69-381)	3,407 (1,458-16,405)	>10,000[a]
Formalin	12,234 (10,291-13,930)	30,680 (27,006-36,318)	60,000
Hydrogen Peroxide	2,209 (2,087–2,353)	3,422 (3,120–3,865)	7,500
Hydrogen Peroxide (Timed Exposure)	3 (0-16)[b]	19 (0-56)[b]	15 (min)
Potassium Permanganate	34 (5-105)	11,152 (2,570-226,414)	100,000

[a] Concentration of free iodine required for 100% mortality is greater than provided in Argentyne
[b] Based on data extrapolation; shortest duration tested was 60 seconds

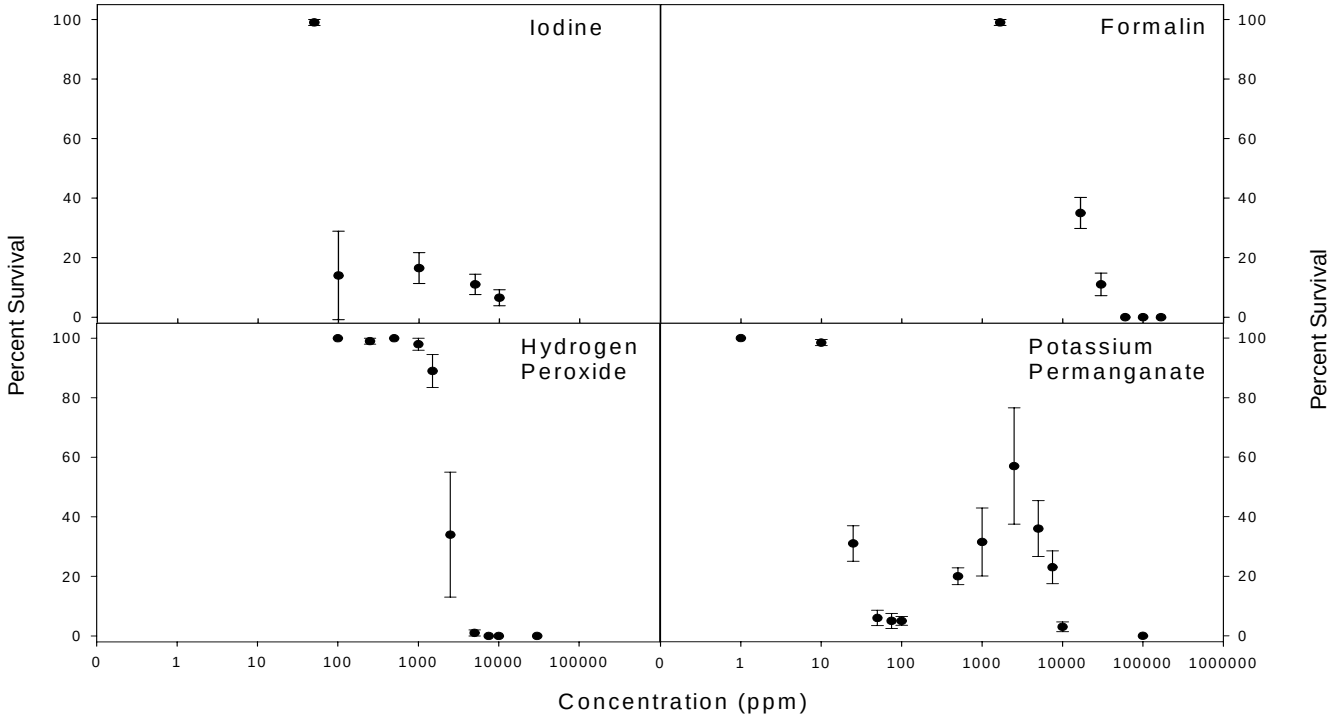

Figure 1: Percent survival of New Zealand mud snails treated with Argentyne (50, 100, 1,000, 5,000, and 10,000 ppm free iodine), formalin (1,667, 16,667, 30,000, 60,000, 100,000, and 166,700 ppm), hydrogen peroxide (100, 250, 500, 1,000, 1,500, 2,500, 5,000, 7,500, 10,000, and 30,000 ppm), and potassium permanganate (1, 10, 100, 1,000, 10,000, and 100,000 ppm) for 15 min. Error bars represent ± 1 SE.

(Continued on page 8)

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(Continued from page 7)

In general, we found that the concentrations of widely used therapeutic chemicals necessary for complete NZMS mortality are greater than the eggs or hatched-fish of most species can tolerate. Therefore, it is not likely that any of these chemicals can be used to effectively remove NZMS that are inadvertently collected during wild egg take operations. Regardless of these results, these chemicals could be used to disinfect hatchery equipment. These chemicals could be used, however, to eradicate snails from de-watered raceways because chemicals would be diluted when the raceways are re-filled. In the end, we feel that proper egg take protocols could be one of the best methods to prevent accidental hatchery introduction. If possible, workers should prevent taking eggs from NZMS infested waters. If it is necessary to take eggs from such areas, water from these lakes and streams should not be transported into hatcheries or should be filtered before use. Also, proper gear disinfection after such collection trips could prevent inadvertent NZMS introduction. Regardless, proper protocol does not guarantee NZMS will not be accidentally introduced. Chemical disinfection of eggs may be the best way to ensure that NZMS are not introduced into other hatcheries.

Randy Oplinger and Eric Wagner

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