New Zealand Mudsnail Containment: Tests of Chemicals, Copper Strips, Fluorochrome Marking and Terrestrial Movement

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Abstract

New Zealand mudsnails (*Potamopyrgus antipodarum*; NZMS) are a non-native species that can have a significant influence the structure of an aquatic community. More research on methods that help prevent the human spread and immigration of the species need to be conducted. We performed four studies in an attempt to increase our understanding of NZMS containment. In the first study, we selected several chemicals that are known to kill NZMS and compared the effectiveness of these chemicals when applied to snails either through a fine spray (0.7-1.1 mL) or immersion in 15-min exposures. We found that copper sulfate (504 and 1,008 mg/L as copper), hydrogen peroxide (30,000 and 60,000 mg/L), Clorox Commercial Solutions 409 Cleaner, Degreaser, and Disinfectant, and Hyamine 1622 (3,880 mg/L) all killed 100 percent of NZMS when applied as a fine spray. This indicated that these chemicals can be used by managers or the public to disinfect equipment that cannot normally be immersed in a chemical. In the second study, we conducted a series of experiments to determine the aptitude and ability of NZMS to move when placed out of the water and found that NZMS were not inclined to move on land, thus indicating that terrestrial movement ability is not a major factor in the design of in-stream barriers. In the third study, we tested a potential immigration barrier constructed using copper strips and found that snails readily crossed strips ≤ 10 cm wide. In the final study, we found that calcein (100 mg/L for 24-48 hr) and tetracycline (300 mg/L for 24-48 hr) can successfully mark NZMS. The calcein mark was visible for at least 5 wks, whereas the tetracycline mark was visible for at least 2 wks. Thus, these chemicals can be used by ecologists and managers to track NZMS movements.

Key Words: calcein, disinfection, invasive species, mollusk, New Zealand mudsnail, *Potamopyrgus antipodarum*, tetracycline, toxicity

Introduction

The introduction of an exotic species can have a multitude of direct and indirect consequences on the other species in a community. New Zealand mudsnails (*Potamopyrgus antipodarum*; syn. *Hydrobia jenkinsi*; NZMS) are non-native to the United States. They were first discovered in Idaho in 1987 and are now distributed throughout western North America (Bowler 1990). In the wild, NZMS can at times attain densities > 500,000 individuals/m^2 (Dorgelo 1987). In some systems, NZMS can constitute > 90 percent of invertebrate production (Hall et al. 2006) and may consume up to 75 percent of gross primary production (Hall et al. 2003). At sufficient densities, NZMS directly influence primary and invertebrate production, and this could presumably have a bottom-up influence on fish populations (Vinson and Baker 2008). In some situations, NZMS have significantly altered aquatic community structure (e.g., Hall et al. 2006). This response can vary; however, as other studies have shown that low NZMS densities can actually facilitate colonization by native invertebrates (e.g., Schreiber et al. 2002).

New Zealand mudsnail populations are now widely distributed, however, despite

their prevalence and potential effect on aquatic communities, our knowledge on gear disinfection and population containment is limited. New Zealand mudsnail populations can expand through transport by animals, e.g., attached to wading birds, in feces of animals that consume NZMS, and immigration. New Zealand mudsnails can also be transported by humans, e.g., through angling equipment, boats, nets, etc. Desiccation, freezing, and chemical disinfection are three of the most effective methods of preventing human-caused spread of NZMS (Richards et al. 2004). Chemical disinfection of equipment is most frequently used by managers because time or weather conditions do not always allow for disinfection through desiccation or freezing. Chemicals such as household ammonia, benzethonium chloride, 409 Cleaner, Degreaser, and Disinfectant, copper sulfate, Pinesol, hydrogen peroxide, potassium permanganate, formalin, and iodine have all been proven to kill a high percentage of NZMS during a relatively short (5-min) exposure duration (Watton and Hawkes 1984; Hosea and Finlayson 2005; Oplinger and Wagner 2009; Schisler et al. 2008). Unfortunately, these previous trials have only fully immersed snails in these chemicals. Situations occur, however, when full immersion is not practical, e.g., with large pieces of equipment or immovable objects, or not wise, e.g., electronic equipment. Whether these chemicals are effective when applied to NZMS in small exposure volumes, such as a spray, remain unknown. Knowing which chemicals are effective when applied as a spray could help prevent inadvertent human-transport of NZMS, reduce the volume of disinfectant used, and decrease cost and negative consequences that chemicals could have on non-target organisms.

Immigration is a second possible method of NZMS population expansion. In-stream barriers offer potential to slow the spread of NZMS. Such barriers could be used to prevent reintroduction after NZMS have been eradicated from an area or can be installed at fish hatcheries or water diversions and treatment plants to prevent NZMS infestation. Practical in-stream snail barriers are still in development and several issues need to be addressed before such barriers can be implemented. Preliminary research has suggested that copper products could be used to prevent upstream movement of NZMS (Myrick and Conlin 2009), but such potential needs further testing. The theoretical basis for use of copper strips lies in the fact that copper is highly toxic to mollusks (Ryder and Bowden 1977, Chandiwana et al. 1987). Numerous copper-based commercial products, e.g., marine anti-fouling paint and garden snail and slug barriers, have been developed (e.g., Schuder et al. 2003, Tatayah et al. 2007) and are readily available to consumers. Also, it is not known if NZMS are capable of amphibious movement. The terrestrial movement abilities of NZMS need to be assessed because if they are capable of leaving water and traveling short distances on land, they could potentially circumvent any installed barriers. Terrestrial movement abilities are not known for any aquatic snail species including NZMS.

Finally, better methods of tracking NZMS immigration and movement need to be developed. The ability to track NZMS movement would provide managers ability to evaluate effectiveness of snail barriers and would allow them to track diel and long-term movements, improving our understanding of NZMS ecology. One of the best methods for tracking movement patterns of NZMS is through the application of a mark. Traditional snail marking techniques, e.g., paint or tags (Yves-Henry and Jarne 2007), are difficult to apply to NZMS because they are a relatively small-bodied species. Marking snails with a fluorochrome dye could be a cost-effective method of marking a large number of snails. Organisms exposed to a fluorochrome incorporate the chemical into growing calcified structures and the mark is visible under ultra-violet light (Muncy et al. 1990). Chemicals such as calcein (1-500 mg/L for 1-48 hr) and tetracycline (20-500 mg/L for 1-48 hr) have effectively marked many

mollusk species (Eads and Layzer 2002, Moran 2000, Moran and Marko 2005, Pirker and Schiel 1993). Less is known about fluorochrome marking of snails, and these dyes have never been tested on NZMS.

The goal of our research was to address some of the previously cited gaps in our knowledge about the disinfection of gear and NZMS population containment. We present results of four studies that we conducted. The first study determined whether six chemicals that are already known to be lethal to NZMS in immersion treatments are similarly toxic when applied as a spray onto snails. The second experiment assessed terrestrial movement ability of NZMS. The third study determined whether copper strips could be used to prevent the upstream movement of NZMS in an artificial stream. Our final study determined whether fluorochrome dyes can be used to mark NZMS and to determine mark retention time. Given the potential threat NZMS have on ecosystem function and biodiversity, we hope that our results improve an understanding of NZMS ecology and increase the number techniques available to disinfect equipment and contain the spread of NZMS.

Methods and Materials

Experiment 1: Spray Test

In this experiment, we determined whether chemicals that are commonly cited to kill NZMS are effective when snails are exposed to the chemical through a fine mist. We tested Hyamine 1622 (1940 and 3880 mg/L, Fluka Chemicals, Steinheim, Germany), copper sulfate (504 and 1008 mg/L as copper, Sigma-Aldrich Chemicals, St. Louis, MO), household ammonia (full strength, Church and Dwight Company, Princeton, NJ), hydrogen peroxide (30,000 and 60,000 mg/L, Thatcher Chemical, Salt Lake City, UT), Lemon-fresh Pinesol (50% dilution and full strength, The Clorox Company, Oakland, CA), Clorox Commercial Solutions 409 Cleaner, Degreaser, and Disinfectant (full strength, The Clorox Company, Oakland, CA), and a control (water). Chemicals were placed in squirt bottles that were adjusted to deliver a fine mist (droplet size not measured; 0.7-1.1 mL/squirt). New Zealand mudsnails were collected by hand from raceways at the Utah Division of Wildlife Resources (UDWR) Loa State Fish Hatchery (Wayne County, Utah), and groups of 25 individuals were transferred to 400 mL plastic beakers. A single chemical squirt was applied to four replicate groups for each chemical. We rinsed the snails three times with hatchery water, and the beaker was filled with water 15 min after exposure. Results from the spray exposures were compared to snails immersed in the chemicals (four replicate groups of 25 NZMS exposed to each chemical) for 15 min. After a 24-hr recovery period, we assessed survival by observing movement under a microscope (Oplinger et al. 2009). Average percent survival was calculated and performance was compared among chemicals using a two-way ANOVA (SAS 1998; main effects: spray vs. immersion and chemical). In our analysis, we treated each combination of chemical and concentration as unique, independent treatments. Data were normalized using an arc-sine transformation, and results were considered statistically significant at $P < 0.05$.

Experiment 2: Terre trial Movement Ability

We conducted three studies to assess the willingness and ability of NZMS to leave water and travel on land. The first two studies were conducted at ambient temperature (12-15 °C) in a Loa State Fish Hatchery building. In the first study, four test arenas were constructed out of plywood (Douglas fir; *Pseudotsuga menziesii*). Each arena consisted of three sections; one horizontal (30 x 15 cm), one vertical (30 x 30 cm; meets horizontal face at 90° angle), and one sloped at a 45° angle (30 x 30 cm; connects at top of vertical section, over-hangs horizontal section). To prevent snails from leaving the arenas, copper stripping was installed along each arena edge. We pumped water down the topside of the 45°

inverted face at a rate of 1.0 L/min. Besides the topside of the inverted face, all other sections of the arena were slightly damp, but contained no standing water. One hundred NZMS were added to the center of the horizontal section of each arena. Once every 24 hrs for 7 days, the number of snails on vertical and inverted surfaces of each arena was counted.

In the second study, four 20 x 30 cm "boxes" were constructed. Each box consisted of a plywood floor. The sides of each box were constructed using different substances; plywood, aluminum, Plexiglas, or concrete (cinder blocks). The sides of each box were 20 cm tall and none of the boxes had a lid. We drilled several holes into the floor of each box to aid in drainage, and vinyl window screen was placed over the holes to prevent snail loss. Boxes were placed into a larger plastic tub that we filled with water to a level just below the box. A pump was placed in the larger tub and an inverted "U" shaped piece of PVC pipe was mounted to this pump. The outlet of this pipe was 10 cm above the center of the box and delivered water at a rate of 5.0 L/ min. Water poured onto a rock, creating a "splashing effect" that kept the sides and the floor of the box wet. One hundred NZMS were added to the floor of the box and the number of snails on the vertical sides of the box was counted daily for 7 days. The experiment was replicated three times on consecutive weeks. New snails were added into the boxes for each replicate trial.

The purpose of the third terrestrial movement study was to assess the effect of temperature and humidity on terrestrial movement (Dainton 1954). For this study, 144 Petri dish test arenas were constructed. Each Petri dish arena consisted of an outer, 100-mm diameter Petri dish with a smaller, 60-mm diameter dish glued into the center. A circular piece of white paper towel was cut and placed into the inner Petri dish. Three temperatures (5, 15, and 22 °C) and four levels of humidity (0, 35, 75, and 98%) were tested. We manipulated temperature through placement of the Petri dish arenas (each in a different building). Temperature was recorded every 15 min during the experiment with a hand thermometer and never deviated more than 1 °C from the target temperature. Humidity was regulated using saturated salt solutions (35%: $MgCl_2 \cdot 6 H_2O$, 75%: NaCl, 98%: K_2SO_4; Winston and Bates 1960). We poured these solutions into the outer Petri dish, and the lid was used to prevent outside conditions from affecting humidity. Prior to use, a hygrometer was used to ensure that we achieved proper humidity. The 0-percent humidity treatment was created using commercial desiccant.

Twelve replicate Petri dish arenas were exposed to each combination of temperature and humidity. Paper towels in six of the twelve replicates were slightly moistened with 0.5 mL of water, whereas the other six remained dry. Snails were allowed to acclimate to conditions for 30 min prior to experiment initiation. Two NZMS were placed in each arena on top of the paper towels. One snail in each dish was larger (2.13 ± 0.18 mm, mean ± SE) than the other (1.21 ± 0.22 mm). The distance that each snail moved was recorded once every 15 min for 3 hr. Four-way ANOVA (SAS 1998; results considered significant at $P < 0.05$) was used to compare the number of times that a snail moved, and the distance moved with temperature, humidity, snail size, and paper towel wetness (moist or dry). Since measurements were only taken every 15 min, the number of moves and movement distance reported was likely conservative.

Experiment 3: Copper Strips

In this experiment, four artificial stream "pairs" were constructed using plastic gutter downspouts. To create the pairs, we mounted two, 2-m long downspout sections parallel to each other and the tail-end of these parallel sections met in a common collection basin. Siphons were used to introduce water at a rate of 1.0 L/ min into each parallel downspout section. Stream pairs were placed in raceways at the Loa State Fish Hatchery and the hatchery water (12 °C) used in this study was filtered (through 100 μm mesh) to prevent snails from being flushed into the artificial streams. Window screen was installed at the tail-end

of the collection basin to prevent snails from being flushed from the stream pairs. A piece of copper strip (roof flashing) was installed 1.0 m downstream from the head of one of the downspout sections of each stream pair (installed on left side of two gutter pairs and on right side on other two). Four different copper strip widths were tested; 2.5, 5.0, 7.5, and 10.0 cm. One hundred NZMS were placed in the collection basin of each stream pair. These snails were freely able to select and move into either parallel stream section (one with and one without copper). Twenty-four hrs after snail addition, we determined the number of snails in each stream section. Also, the number of snails upstream of the copper strips (or line drawn 1.0 m downstream from head in control streams) was counted. The experiment was repeated on 3 consecutive days, allowing us three replicate runs at each strip width. Snails were replaced between days. Two-way ANOVA (SAS 1998; results considered significant at $P < 0.05$) was used to compare snail numbers between the gutter sections (copper vs. no copper) and among copper strip widths. We also determined whether copper strips significantly elevated water copper concentrations by collecting water samples. The copper concentration in these samples was determined using an atomic absorption spectrophotometer (Thermo Electron Solaar S2 AA; P. Hole, Utah State University, personal communication).

Experiment 4: Use of Calcein and Tetracycline to Mark New Zealand Mudsnails

The purpose of this experiment was to determine whether calcein or tetracycline can be used to mark snails. To do this 15 6-L plastic tubs were used. Each tub was filled with 4 L of water and maintained at ambient temperature (12-14 °C). Two hundred and fifty NZMS were placed into each tub. Calcein was added to six of the tubs at a concentration of 100 mg/L and tetracycline was added to six more tubs at a concentration of 300 mg/L. The remaining three tubs did not receive any chemical and served as controls. We exposed snails in three tubs from each chemical treatment to chemical for 24 hr, and the remaining tubs were exposed for 48 hr. After treatment, the chemical solution was removed from the tubs and snails were thoroughly rinsed and the tubs were re-filled with hatchery water. We added 5 g of fresh, NZMS-free algae. A sample of 25 snails was removed from each tub 24 hr, and 1, 2, 3, 4, 5, and 8 wks after the end of chemical treatment. Samples were preserved by freezing and stored in the dark to prevent photo-degradation. Snails were observed under a fluorescence microscope with excitation wavelengths ranging from 460-530 nm and the proportion of snails with a distinct fluorochrome mark was determined. The presence of a mark was verified by comparing the treated snails with NZMS from the control tubs.

Results

Experiment 1: Spray Test

New Zealand mudsnail survival varied among the chemicals tested (Fig. 1; $F_{10,66}$ 2735.0, $P < 0.01$). One hundred percent of snails treated with both spray and immersion with copper sulfate (both concentrations), hydrogen peroxide (both concentrations), Commercial 409, and the highest concentration of Hyamine 1622 (3880 mg/L) died. We observed a significant application method (spray vs. immersion) x chemical interaction ($F_{10,66} = 925.0$, $P < 0.01$). This interaction was driven by Pinesol. None of the snails immersed in Pinesol survived, but survival among NZMS sprayed with Pinesol was 96-98 percent. Overall, 11.0 ± 1.9 percent of NZMS exposed to a spray of household ammonia survived. All of the chemicals produced 100-percent mortality in the immersion trials.

Experiment 2: Terrestrial Movement Ability

No snails left the horizontal section of arenas used in the angle experiment. Similarly, we found no snails on the box sides in the second experiment. Both studies lasted 7 days, and during these trials no snails traveled > 25 mm from their starting point. These results show that NZMS have little aptitude to move when out of the water.

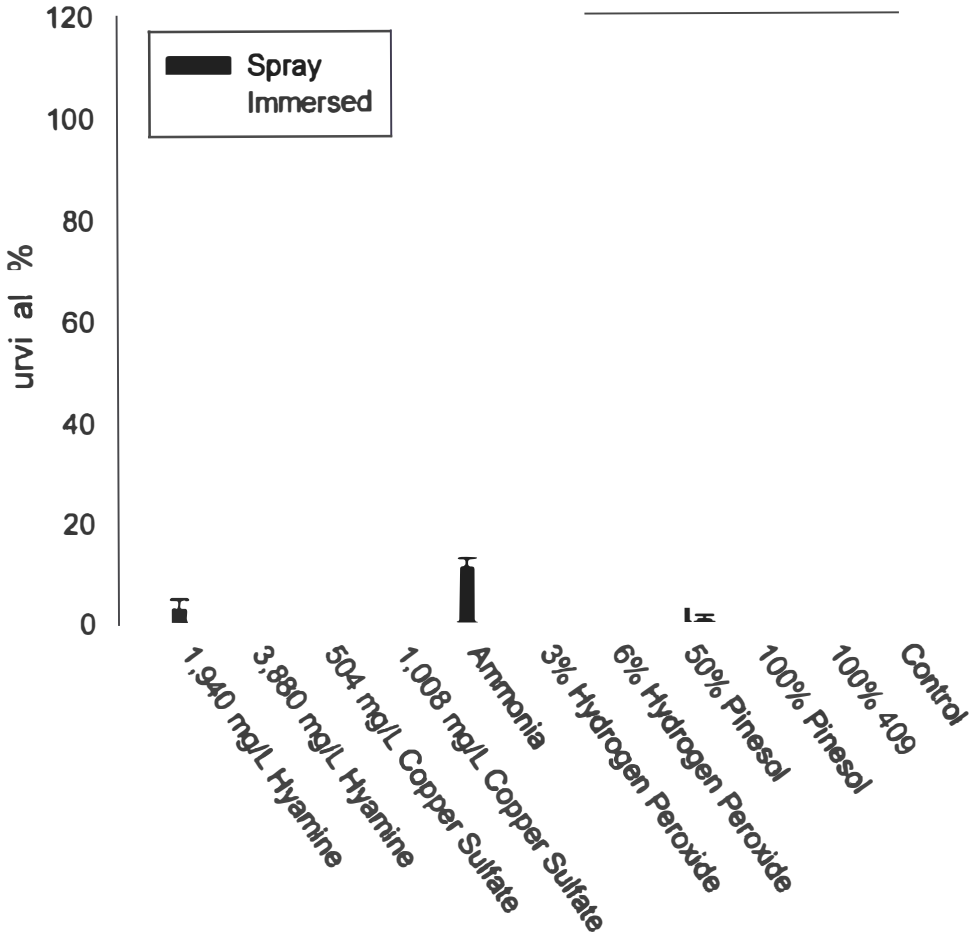

Figure 1. Percent survival of New Zealand mudsnails that were either exposed to spray (0.7-1.1 mL misted on dry snails; black bars) or immersed (grey bars) in a variety of chemicals for 15 min. Error bars represent ± 1 SE of the mean.

In the temperature and humidity study, no snails on the dry paper towels moved. Consequently, these treatments were excluded from further analysis. Among the snails on the moistened paper towels, the total distance moved increased with temperature (Fig. 2; $F_{2,120} = 19.8$, $P < 0.01$). A Tukey's Multiple Comparison Test showed that distance moved over 3 hrs did not vary among snails at 15 and 22 °C ($F_{1,3} = 6.4$, $P = 0.07$); however, movement at both temperatures was statistically greater than the lowest temperature (5 °C; both $P < 0.01$). Larger snails moved a greater distance than smaller snails (on average, 13.3 ± 2.5 mm more; $F_{1,120} = 32.0$, $P < 0.01$). Similarly, the total number of moves recorded over the 3-hr trial varied with temperature ($F_{2,120} = 27.6$, $P < 0.01$). Total number of recorded moves over 3 hrs did not vary among snails at 15 and 22 °C ($F_{1,3} = 1.6$, $P = 0.49$); however, movement at both temperatures was greater than the lowest temperature (5 °C; both $P < 0.01$). On average, 1.5 ± 0.3 more moves were recorded for larger snails than smaller snails ($F_{1,120} = 37.5$, $P < 0.01$). Humidity had no effect on distance moved (Fig. 2; $F_{3,120} = 1.2$, $P = 0.33$) or the number of times they moved ($F_{3,120} = 0.6$, $P = 0.62$). Only 25.3 percent of the snails utilized in the trial, not including snails on dry paper towels, moved, and the maximum distance any snail moved over 3 hrs was 113 mm, e.g., a large snail on a moist towel at 22 °C and 0 percent humidity. Most observed movement occurred in the first hour of the trial. A linear regression between distance moved during each 15-min observation period and time showed a significant decreasing trend ($F_{1,6} = 7.3$, $P = 0.04$; 18.0 ± 5.4 mm average in 1st hr, 16.6 ± 5.0 mm in 2nd hr, 10.2 ± 3.0 mm in 3rd hr).

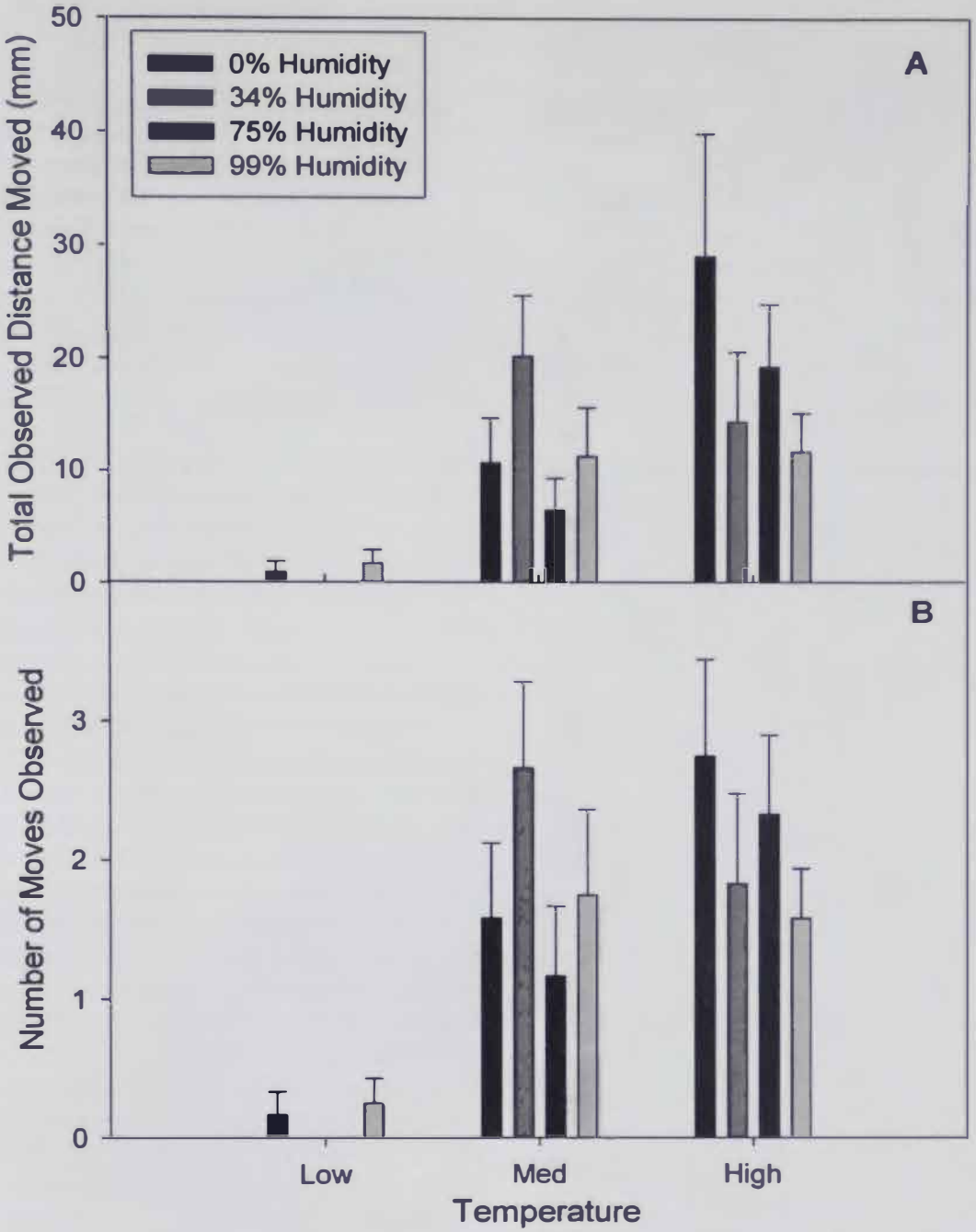

Figure 2. Average distance moved (mm; top panel) and number of individual moves recorded (bottom panel) during three hours of observation of New Zealand mudsnails. Results are separated by temperature (low =5 °C, medium = 15 °C, high = 22 °C) and humidity. Movement was observed on snails out of the water, however, all snails were placed on a slightly moistened substrate. Results from snails on dry substrates are not shown in this figure. Error bars represent ± 1 SE of the mean.

Experiment 3: Copper Strips

New Zealand mudsnails were found across the copper strips in all 12 replicate trials. The number of snails found to enter a stream section did not vary with the presence of copper or by strip width (Table 1; both $P > 0.23$). Snails were able to cross all four strip widths tested. The number that crossed did not vary with strip width ($F_{3,16} = 3.70$, $P = 0.07$). Copper concentrations in all of the water samples were below the detection limits of the spectrophotometer (0.008 mg/L). Therefore, any potential increase in soluble copper concentrations due to the strips was trivial.

Table 1. Comparison of New Zealand mudsnail numbers, i.e., selection preference, between troughs with and without copper strips of various widths. Numbers in parentheses represent ± 1 SE of the mean.

Copper Strip Width (cm)	Gutter Section Type	Average Number Above Line	Average Total Number in Gutter Section
2.5	Copper	27.7 (3.5)	45.3 (2.2)
5.0	Copper	27.0 (2.5)	42.0 (2.5)
7.5	Copper	27.0 (3.0)	47.7 (2.7)
10.0	Copper	28.7 (2.9)	53.0 (1.5)
2.5	No Copper	34.0 (1.7)	43.7 (1.2)
5.0	No Copper	37.7 (4.6)	49.0 (2.1)
7.5	No Copper	27.0 (2.6)	44.3 (2.0)
10.0	No Copper	27.7 (1.2)	44.7 (1.9)

Experiment 4: Use of Calcein and Tetracycline to Mark New Zealand Mudsnails

Both calcein and tetracycline successfully produced a visible mark on NZMS shells. The coloration and positioning of these marks varied by chemical. Calcein tended to produce a dark, "electric green" mark positioned near the first whorls on the middle section of the shell. This mark was most visible when the shell was viewed from the sides or dorsally and was less visible on the ventral side. Calcein marks were distinct and visible on 100 percent of snails collected within the first five weeks after marking. The calcein mark was observed on 82 out of 150 snails (55%) collected for the 8-wk sample. We observed no difference in the intensity of the mark among snails that were treated for either 24 or 48 hrs. Tetracycline produced a lighter, "electric yellow-green" mark that was most visible on the aperture. This mark was visible on all snails collected in samples taken 1-2 wks after marking. The mark was less visible among snails that were collected 3 wks after marking (39%, 58 out of 150 snails) and was not observed on any snails in the 4-, 5-, and 8-wk samples. Again, we observed no difference in mark intensity among snails that were held in chemical for either 24 or 48 hrs. The control (non-treated) NZMS had naturally fluorescing pigments in their operculum tissue that glowed an "electric yellow-green" color that was similar to the tetracycline mark. In smaller snails, this natural fluorescence was visible through the aperture of the shell and could be easily confused as a tetracycline mark. Survival of the marked snails 24 hrs after chemical treatment was 100 percent; therefore, the chemicals did not affect survival.

Discussion

In some ecosystems, establishment of NZMS has had profound direct and indirect consequences on community structure and function. Unfortunately, while potential problems associated with NZMS colonization are well documented, methods of gear disinfection and population containment are poorly studied. More tools need to be developed for use by managers to prevent the spread of NZMS. Our results show that hydrogen peroxide, Hyamine 1622, copper sulfate, and Commercial 409 all kill 100 percent of NZMS when applied to air-exposed snails as a mist. Thus, smaller chemical volumes than previously tested (Watton and Hawkes 1984; Hosea and Finlayson 2005; Oplinger and Wagner 2009) are effective at killing NZMS. Spray application of these chemicals can be used by managers and the public as an alternative method for gear disinfection. In addition, spray application reduces chemical costs, the quantity of chemical necessary for disinfection, the amount of chemical released into the environment and potentially minimizes negative effects on non-target organisms. Spray application of chemical could also allow for disinfection of large, e.g., boats and vehicles, and sensitive, e.g., electronics or vehicle

interiors, equipment that would normally be difficult to immerse in chemical. Since the application of a spray increases the number of items that can be disinfected, more items can be treated, reducing the probability of accidental human spread of NZMS. We know that some of these chemicals can have adverse effects on fabrics (Hosea and Finlayson 2005); even though these chemicals may be effective at killing snails, more material safety tests should be conducted before implementing use of some chemicals for disinfection.

Pinesol and household ammonia were the only two chemicals that did not have comparable performance in both the spray and immersion trials. Why these chemicals were less effective as a spray remains unclear. The component of Pinesol that is lethal to NZMS is not known; however, alcohol ethoxylate surfactants are the active antibacterial ingredient in Pinesol, (10,000-50,000 ppm; Material Safety Data Sheet). Alcohol ethoxylate surfactants are known to degrade lipid membranes (Wong et al. 2004, Lizotte et al. 1999). The active ingredient quantities provided in a fine mist probably are insufficient to produce significant membrane damage in NZMS. Limited information regarding the toxicity of alcohol ethoxylate surfactants on mollusks is available; however, it appears that Pinesol is the least toxic of the chemicals tested. Previous studies have shown that fish eggs are tolerant of continuous alcohol ethoxylate surfactant exposures of 10 mg/L for up to 56 d (Wong et al. 2004, Lizotte et al. 1999). Why household ammonia was less effective when administered as a spray is also unknown. Ammonia is highly toxic to NZMS, with reported EC_{50} values ranging between 0.31 and 0.85 mg/L (Watton and Hawkes 1984, Hickey and Vickers 1994). Again, the spray possibly provided an insufficient chemical quantity to kill 100 percent of NZMS. Even though spray application reduces the quantities of chemical required for disinfection, the effects of these chemicals on non-target organisms should be considered. We know that Hyamine 1622 (1940 mg/L), Commercial 409, and household ammonia are all toxic to fish eggs, and the active ingredients could have negative effects on other non-target organisms (Burkhalter and Kaya 1977, Daniels et al. 1987, Oplinger and Wagner 2009).

Chemical disinfection of equipment is one potential method of preventing further spread of NZMS. Construction of in-stream barriers designed to prevent upstream movement of NZMS is another possible method. One concern with construction of such barriers is that NZMS could potentially leave the water and crawl over-land, around any installed barriers although such potential of terrestrial movement has never been assessed. We investigated this potential and found that NZMS have little ability or aptitude to move when placed out of the water. In the first two trials with the angled plywood structures and boxes we did not measure fine-scale movement. Thus, some snails possibly moved a considerable distance, but we did not note any such movement since they did not leave the vicinity where initially placed. Since NZMS position was only recorded once every 24 hrs, some snails may have left the location where they were initially placed and traveled a considerable distance but happened to be near where they were placed at the time when movement was recorded. This, however, is not likely since movement was recorded for 7 days. Fine-scale movements were measured in the temperature and humidity experiment. With the exception of one snail that moved 113 mm, every snail used in this study traveled < 50 mm, which suggested that snails were reluctant to move and did not move quickly when placed out of the water. Snails that did move during this study tended to do so in an irregular pattern (R.W. Oplinger, personal observation), suggesting that NZMS are not able to travel to a pre-selected destination when out of the water. We observed greater terrestrial movement at higher temperatures, which is consistent with terrestrial snail and slug species (Dainton 1954). Additionally, terrestrial and aquatic snails are ectothermic and their metabolic rate

varies with temperature. We observed no increase in terrestrial movement of NZMS with increased humidity; this differed from observations of terrestrial species by Bailey (1975) and Dainton (1954). That aquatic snails did not react to humidity in a similar manner as terrestrial snails was somewhat surprising; however, humidity is not a factor in the aquatic environment, so aquatic snails may not have evolved an ability to detect and respond to changes in humidity. Only snails placed on wet paper towels moved during this experiment suggesting that if managers want to design NZMS migration barriers, steps should be taken to ensure the land surrounding the barrier is kept dry NZMS may only be capable of moving over wet surfaces. Wet substrates are indicative of the presence of nearby water and snails may move over wet surfaces to seek out water deep enough for immersion. The majority of movement we observed occurred in the first hour. New Zealand mudsnails are susceptible to freezing and desiccation (Richards et al. 2004). To prevent this from happening, NZMS may move more when initially placed in the air in an attempt to seek water. They then may decrease movement with time to conserve energy and limit exposure to predators. Alternatively, cessation of movement and operculum withdrawal may be a mechanism that NZMS use to conserve water and prevent desiccation. The 3-hr observation period used in this study is relatively short, however, in a small preliminary trial that lasted 15 hrs, no movement was observed after the third hour of observation (R.W. Oplinger, unpublished data).

The installation of copper strips is one potential in-stream barrier that could be used by managers to prevent the immigration NZMS into new territories. Such barriers could also be used to prevent reintroduction after NZMS have been eradicated from an area or can be installed at fish hatcheries or water diversions and treatment plants to prevent NZMS infestation. Copper is highly toxic to mollusks (Ryder and Bowden 1977, Chandiwana et al. 1987) and strips have been used to confine terrestrial mollusk species (Symondson 1993, Tatayah et al. 2007). It has been assumed that copper strips could be used to prevent the movement of NZMS; however, effectiveness of such strips has never been assessed. The assumption that copper strips could be effective at preventing the upstream movement of NZMS is based on research that has shown that copper is toxic to NZMS. Studies have reported NZMS 48-hr EC_{50} values for copper sulfate ranging between 0.058 and 0.112 mg/L (Watton and Hawkes 1984). Chemicals that release copper ions into the water, e.g., copper sulfate, have been successfully used to disinfect equipment (Hosea and Finlayson 2005). Despite the high toxicity of copper, we found that NZMS were not able to detect the presence of copper in our artificial streams and freely crossed the test strips. These results indicate that higher concentrations of copper ions or wider strips need to be present to deter NZMS movement. The strips that we used were new, and if these strips were allowed to age prior to use, pitting and corrosion possibly would have increased copper release, improving strip effectiveness. Alternatively algae and biofilm growth may occur over time, forming a protective layer that may reduce copper exposure to the water and strip effectiveness. Other forms of copper such as mesh and copper-based marine anti-fouling paint may prove superior as in-stream barriers than solid copper strips (C. Myrick, Colorado State University, personal communication). In the future, wider strips need to be tested. One limitation of copper strips is that they do not prevent NZMS transport by other animals, e.g., birds, beavers, etc.. Barriers that prevent transport by these organisms also need to be evaluated.

Regardless of the effectiveness of any installed barriers, successful methods of tracking NZMS movement are essential to managers and ecologists to help predict population expansion and potential impacts on native species. Due to their small size, however, traditional snail marking methods such as paints or marks that are glued to the shell are difficult to apply to NZMS. We found that fluorochromes calcein and

tetracycline can be used to mark NZMS. Exposure durations that we tested were on the longer end of what has been tested with other mollusk species (Eads and Layzer 2002, Moran 2000, Day et al. 2005, Pirker and Schiel 2003). Calcein concentrations that we tested fell at the lower end of the range of what has been previously tested with mussels (Kaehler and McQuaid 1999, Moran 2000, Moran and Marko 2005). Tetracycline concentrations that we used were typical of other studies (Eads and Layzer 2002). Shorter exposure durations or concentrations that differ from what we tested possibly could also successfully mark NZMS. We found that calcein produced a brighter, longer lasting, and easier to observe mark than tetracycline. No mortality was observed among snails marked with both fluorochromes. Our results with NZMS differed from those that have demonstrated low survival in other mollusks treated with tetracycline (Kaehler and McQuaid 1999). We observed 100-percent mark retention among snails marked with calcein for the first 5 wks after treatment, and for the first 2 wks among tetracycline marked individuals. Since it has a longer mark retention time, we recommend the use of calcein as a fluorochrome dye for NZMS.

The spread of alien species is considered one of the biggest threats to aquatic biodiversity in western North America (Wilcove et al. 1998). New Zealand mudsnails are one of the most common aquatic invasive species in the region. Our research goal was to investigate useful methods to improve our ability to disinfect equipment and contain the spread of the species. Our results demonstrated that spray application of Hyamine 1622, Commercial 409, copper sulfate, and hydrogen peroxide all kill 100 percent of NZMS. These results show that it is possible to disinfect equipment that normally cannot be treated through immersion. Increasing the number of items that can be disinfected could help reduce the spread of NZMS. We also demonstrated that NZMS have little aptitude or ability to travel outside of the water. This suggests that when migration barriers are installed, minimal effort needs to be invested in preventing the terrestrial movement of NZMS. We also tested copper strips as potential in-stream barriers and found that at least at the widths tested, that copper strips were not effective, and that alternative barriers need to be developed and tested. Finally, we demonstrated that both calcein and tetracycline can be used as fluorochrome dyes to batch mark NZMS. This batch-marking process can be used to help evaluate the effectiveness of in-stream barriers and in studies that track NZMS movement patterns. More research on NZMS containment and gear disinfection is necessary; however, the results from our studies improve our understanding of these topics and can be applied by managers and ecologists.

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Literature Cited

Bailey, S. E. R. 1975. The seasonal and daily patterns of locomotor activity in the snail *Helix aspersa* Müller and their relation to environmental variables. Proceedings of the Malacological Society of London 41: 415-428.

Bowler, P. A. 1991. The rapid spread of the freshwater hydrobiid snail *Potamopyrgus antipodarum* (Gray) in the middle Snake River, southern Idaho. Proceedings of the Desert Fishes Council 21:173-182.

Burkhalter, D. E. and C. M. Kaya. 1977. Effects of prolonged exposure to ammonia on fertilized eggs and sac fry of rainbow trout (*Salmo gairdneri*). Transactions of the American Fisheries Society 106: 470-475.

Chandiwana, S. K., J. Ndamba, O. Makura, and P. Taylor. 987. Field evaluation of controlled release copper glass as a molluscicide in snail control. Transactions of the Royal Society of Tropical Medicine and Hygiene 81:952-955.

Dainton, B. H. 1954. The activity of slugs I: the induction of activity by changing temperatures. Journal of Experimental Biology 31: 165-187.

Daniels, H. V., C. E. Boyd, and R. V. Minton. 1987. Acute toxicity of ammonia and nitrate to spotted seatrout. Progressive Fish-Culturist 49: 260-263.

Day, R. W., M. C. Williams, and G. P. Hawkes. 1995. A comparison of fluorochromes for marking abalone shells. Marine and Freshwater Research 46: 599-605.

Dorgelo, J. 1987. Density fluctuations in populations (1982-1986) and biological observations of *Potamopyrgus jenkinsi* in two trophically differing lakes. Hydrobiological Bulletin 21: 95-110.

Eads, C. B. and J. B. Layzer. 2002. How to pick your mussels out of a crowd: using fluorescence to mark juvenile freshwater mussels. Journal of the North American Benthological Society 21:476-486.

Hall, R. O., J. L. Tank, and M. F. Dybdahl. 2003. Exotic snails dominate nitrogen and carbon cycling in a highly productive stream. Frontiers in Ecology and the Environment 1:407-411.

Hall, R. O., M. F. Dybdahl, and M. C. Vanderloop. 2006. Extremely high secondary production of introduced snails in rivers. Ecological Applications 16:1121-1131.

Hickey, C. W. and M. L. Vickers. 1994. Toxicity of ammonia to nine native New Zealand freshwater invertebrate species. Archives of Environmental Contamination and Toxicology 26:292-298.

Hosea, R. C. and B. Finlayson. 2005. Controlling the spread of New Zealand mudsnails on wading gear. Administrative Report 2005-02 California Fish and Game, Office of Spill Prevention and Response, Sacramento.

Kaehler, S. and C. D. McQuaid. 1999. Use of the fluorochrome calcein as an in situ growth marker in the brown mussel *Perna perna*. Marine Biology 133:455-460.

Lizotte, R. E., D. C. L. Wong, P. B. Dorn, and J. H. Rodgers. 1999. Effects of a homologous series of linear alcohol ethoxylate surfactants on fathead minnow early life stages. Archives of Environmental Contamination and Toxicology 37: 536-541.

Moran, A. L. 2000. Calcein as a marker in experimental studies newly-hatched gastropods. Marine Biology 137:893-898.

Moran, A. L. and P. B. Marko. 2005. A simple technique for physical marking of larvae of marine bivalves. Journal of Shellfish Research 24:567-571.

Muncy, R. J., N. C. Parker, and H. A. Poston. 1990. Inorganic chemical marks induced in fish. American Fisheries Society Symposium 7:541-546.

Myrick, C. and S. Conlin. 2009. Copper-based substrates as potential barriers to New Zealand mudsnails. Western Division of the American Fisheries Society Meeting Abstract. http://www.aznmfishsoup.org/wdafs09/docs/2009WDAFSabstracts.pdf. Accessed 16 July, 2009.

Oplinger, R. W. and E. J. Wagner. 2009. Toxicity of Common Aquaculture Disinfectants to New Zealand Mudsnails and Mudsnail Toxicants to Rainbow Trout Eggs. North American Journal of Aquaculture 71:229-237.

Oplinger, R. W., P. Brown, and E. J. Wagner. 2009. Effect of Sodium Chloride, Tricaine Methanesulfonate, and Light on New Zealand Mudsnail Behavior, Survival of Snails Defecated from Rainbow Trout, and Effects of Epsom Salt on Snail Elimination Rate. North American Journal of Aquaculture 71:157-164.

Pirker, J. G. and D. R. Schiel. 1993. Tetracycline as a fluorescent shell-marker in the abalone *Haliotis iris*. Marine Biology 116:81-86.

Richards, D.C., P. O'Connell, and D.C. Shinn. 2004. Simple control method to limit the spread of New Zealand mudsnail *Potamopyrgus antipodarum*. North American Journal of Fisheries Management 24:114-117.

Ryder, T. A. and I. D. Bowden. 1977. The slug foot as a site of uptake of copper molluscicide. Journal of Invertebrate Pathology 30:381-386.

SAS Institute. 1998. SAS/STAT user's guide version 8.0, volume 1. SAS Institute, Cary, NC.

Schisler, G.J., N.K.M. Vieira, and P.G. Walker. 2008. Application of household disinfectants to control New Zealand mudsnails. North American Journal of Fisheries Management 28:1172-1176.

Schreiber, E. S. G., P. S. Lake, and G. P. Quinn. 2002. Facilitation of native stream fauna by an invading species? Experimental investigations of the interaction of the snail, *Potmopyrgus antipodarium* (Hydrobiidae) with native benthic fauna. Biological Invasions 4:317-325.

Schuder, I., G. Port, and J. Bennison. 2003. Barriers, repellents and antifeedants for slug and snail control. Crop Protection 22:1033-1038.

Synmondson, W. O. C. 1993. Chemical confinement of slugs: an alternative to electric fences. Journal of Molluscan Studies 54:259-261.

Tatayah, R. V. V., J. Malham, and P. Haverson. 2007. The use of copper strips to exclude invasive African giant land-snails *Achatina* spp. from echo parakeet *Psittacula eques* nest cavities, Black River Gorges National Park, Mauritius. Conservation Evidence 4:6-8.

Vinson, M. R. and M. A. Baker. 2008. Poor growth of rainbow trout fed New Zealand mudsnails *Potamopyrgus antipodarum*. North American Journal of Fisheries Management 28:701-709.

Watton, A. J., and H. A. Hawkes. 1984. The acute toxicity of ammonia and copper to the gastropod *Potamopyrgus jenkinsi* (Smith). Environmental Pollution (Series A) 36:17-29.

Wong, D. C. L., R. J. Toy, and P. B. Dorn. 2004. A stream mesocosm study on the ecological effects of a C_{12-15} linear alcohol ethoxylate surfactant. Ecotoxicology and Environmental Safety 58: 173-186.

Yves-Henry, P. and P. Jarne. 2007. Marking hard-shelled gastropods: tag loss, impact on life-history traits, and perspectives in biology. Invertebrate Biology 126:138-15

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